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# MICROBIAL DEGRADATION/ALTERATION OF CARBONACEOUS SPECIES IN A COPPER/MOLYBDENUM CONCENTRATE

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Final Report

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## 1.0 EXECUTIVE SUMMARY

The molybdenum flotation circuit of a major Canadian copper-molybdenum producer has had numerous difficulties separating a hydrocarbon mineral (gilsonite) from molybdenite using conventional metallurgical methods. Biotechnology was explored as an economically viable adjunct to conventional metallurgical processing. The research centered on isolating suitable microorganisms which can effectively degrade fractions of the hydrocarbon material present in a hydrocarbon rich molybdenum ore concentrate. This report summarizes the Phase I project.

A literature review pertaining to hydrocarbon oxidizing microorganisms was completed. The objective of the literature review was to determine an experimental strategy for selection of microorganisms having the ability to degrade many types of carbonaceous material.

A mixed culture of microorganisms have effectively degraded the asphalt fraction from a hydrocarbon-rich molybdenum ore concentrate. These microorganisms were originally isolated from waste petroleum activated sludge reactors from the Petro Canada and Esso oil refineries at Clarkson and Sarnia respectively.

Bacterial strains were isolated and enriched on the organically extracted asphalt fraction of the hydrocarbon material from the ore concentrate. Bacteria were propagated in both solid and liquid media with nutrient salts supplementation. The bacterial genera Pseudomonas sp., Flavobacterium sp., Mycobacterium sp. and Arthrobacter sp. were the most

predominant strains present in the isolated mixed cultures. On occasion, protozoa were present in cultures growing on molybdenum concentrates. The protozoan was tentatively identified as Bodo sp.

A bacterial surfactant was produced which caused the suspension of ore particles, preferentially holding carbonaceous particles in solution. The effect may enable carbonaceous material to be removed from reactor vessels with little or no loss of molybdenite.

Infrared spectrophotometric studies demonstrated the oxidation of hydrocarbons due to the bacterial treatment. Gas chromatographic analyses also showed the disappearance of high molecular weight asphaltene hydrocarbons from the treated ore concentrate.

It is believed that the bacterial treatment liberates the "sticky" asphalt-like hydrocarbons from other ore particles. Subsequent cycloning of bacterially treated ore concentrates through one-inch diameter cyclones showed improvements in carbon rejections compared to the Mine's cycloning data. Over 90% carbon rejection was obtained at a feed density of 12.5% solids from the bacterially treated concentrate. The Mine's cycloning runs have achieved only 80% carbon rejection at the same feed density.

Semi-continuous bacterial treatment of the ore concentrate showed increased rates of hydrocarbon removal. Up to 26% of the carbon was removed when set up in a semi-continuous decantation system over a 14 day period. Batch treatment studies on both bench and pilot plant scale showed negligible decreases in carbon content.

## RÉSUMÉ GÉNÉRAL

Le circuit de flottation du molybdène, à la mine Island Copper, près de Port Hardy, en Colombie-Britannique, a éprouvé de nombreuses difficultés pour séparer un minéral hydrocarbure (gilsonite) du molybdénite en utilisant des méthodes métallurgiques conventionnelles. On a, par conséquent, utilisé des méthodes biotechnologiques comme solution économique au processus métallurgique conventionnel. Ce rapport résume les résultats d'une recherche effectuée dans le cadre de la phase I de notre projet axé sur l'isolation de micro-organismes pouvant conduire efficacement aux fractions dégradables de l'hydrocarbure présent dans un concentré de minerai riche en molybdène.

Une étude complète fut effectuée sur les micro-organismes contenant des oxydants d'hydrocarbures. L'objectif de cette étude était de déterminer une stratégie expérimentale afin de sélectionner les micro-organismes possédant la possibilité de dégrader de nombreux types de matières carbonées.

Une culture mixte de micro-organismes a réussi efficacement à dégrader la fraction bitumique d'un concentré de minerai à base de molybdène riche en hydrocarbure. Au départ, ces micro-organismes ont été isolés des réacteurs de cambouis activés par les déchets prétoliers aux raffineries Pétro-Canada, de Clarkson et Esso de Sarnia.

Les souches bactériennes furent isolées et enrichies avec la fraction bitumière organiquement extraite de l'hydrocarbure du concentré de minerai. Les bactéries furent associées sous une forme solide et liquide à un supplément de sel nutritif. Les genres bactériens Pseudomonas, Flavobacterium, Mycobacterium et

Arthrobacter s'avérèrent les souches les plus prédominantes dans les cultures associées isolées.

Un tensioactif bactérien fut produit, ce qui permit la suspension de particules de minerai contenant, de préférence, des particules carbonées dans une solution. Cette solution permet de supprimer les matières carbonées des cuves du réacteur avec peu ou, sinon, aucune perte de molybdène.

Des études spectrophotométriques aux infra-rouges ont démontré l'oxydation d'hydrocarbures suite au traitement bactérien. Des analyses chromatographiques en phase gazeuse ont également indiqué la disparition d'hydrocarbures asphaltènes de masse moléculaire élevée du concentré de minerai traité.

On pense que le traitement bactérien élimine les hydrocarbures du type bitumeux et "collant", ce qui permet de les séparer des autres particules de minerai. Un traitement par cyclone des concentrés de minerai traités sur une base bactérienne, de l'ordre de cyclone d'un diamètre d'un pouce, permet d'améliorer la réjection carbonique par rapport aux données par cyclone de Island Copper. Une réjection carbonique de plus de 90% fut obtenue avec une alimentation métrique de 12,5% de solides à partir des concentrés traités bactériennement. Le traitement par cyclone Island Copper n'a permis d'obtenir qu'une réjection carbonique de 80% avec la même alimentation densimétrique.

Un traitement bactérien sur une base semi-permanente de concentré de minerai a permis d'obtenir des taux plus élevés d'extraction d'hydrocarbures. Jusqu'à 26% du carbone furent enlevés après avoir placé ce minéral dans un système de décantation semi-continu pendant 14 jours. Les analyses de traitement par groupe à l'échelle banc d'essai et usine pilote ont indiqué des diminutions très négligeables du contenu en carbone.

## 2.0 INTRODUCTION

Gilsonite, a hydrocarbon mineralization which contaminates approximately 20% of the ore body of a major copper-molybdenum producer has caused numerous problems in the molybdenum concentrator since plant start-up in 1971. The hydrocarbon affects the molybdenum flotation circuit contaminating the final molybdenum concentrate decreasing the final grade. Revenue losses are the net result of the hydrocarbon contamination.

Extensive studies have been initiated to alleviate the hydrocarbon problem and to date, no feasible selective separation method has been found to effectively separate the hydrocarbon from the concentrate. Of the separation methods tested, the use of one-inch diameter cyclones proved practical, although problems still occurred when hydrocarbon contamination in the feed was high. Consequently, the cycloning efforts were discontinued.

Conventional metallurgical methods of treating the ore to separate hydrocarbon have been exhausted and other methods are being sought to help solve the problem. The use of microorganisms to treat the hydrocarbon-rich ore is being evaluated as an adjunct to present metallurgical practice. It is thought that the "sticky" asphalt-like hydrocarbon causes fine ore particles to aggregate, resulting in a difficult, if not impossible separation of hydrocarbon from molybdenite.

Hydrocarbon oxidizing microorganisms can be used to alter the composition of the hydrocarbons in the ore concentrates, rendering them free from other ore particles, and making them less "sticky" than the original form. Ore concentrates processed in such a way could be treated by conventional gravity methods (i.e. cycloning, tabling, or heavy liquid separation) to enhance the separation of hydrocarbon from molybdenum, producing a higher grade of molybdenum concentrate.

The goal of this research program was to isolate microorganisms having the ability to alter the hydrocarbon in the molybdenum concentrate such that conventional metallurgical methods can be used to separate the carbonaceous fraction from the molybdenite with increased efficiency. Bacterial growth parameters were optimized to allow for maximal degradation of hydrocarbon. Following bench scale work, the bacterial treatment of ore concentrate was transferred to the pilot plant for a cyclone separation of hydrocarbon from the molybdenite.

This report summarizes the work completed to date, on the bacterial treatment of molybdenite concentrate and discusses the work that will be performed in the immediate future.

## 3.0 LITERATURE REVIEW

### 3.1 Objective

The objective of the review is to obtain and assess all literature relevant to hydrocarbon degrading microorganisms. Microorganisms from all habitats will be studied. The scope of the search will include academic and industrial studies on natural ecosystems, oil spills, and waste treatment processes. Organism selection and growth optimization techniques will be examined. Products of hydrocarbon metabolism will also be studied. The culmination of this literature review will be an experimental strategy to use microorganisms to remove hydrocarbons from contaminated concentrates.

## 3.2 Findings

A computer-assisted literature search was performed. Key articles and secondary references were then consulted and are listed in the bibliography (Appendix I).

### 3.2.1 Molybdenum Oxidation by Microorganisms

In order to solve the hydrocarbon contamination problem in the Mine's circuit, two solutions have been proposed which utilize microbiological oxidation phenomena:

1. To utilize hydrocarbon-oxidizing microoganisms to degrade and/or oxidize the hydrocarbon material so that it harbours alternate surface properties, resulting in a cleaner flotation process.

2. To oxidize the surface of the molybdenite so that it may be selectively depressed or becomes non-floatable. The hydrocarbons can subsequently be floated off and the molybdenum can be upgraded after hydrocarbon removal.

A number of sulfur-oxidizing bacteria have the ability to oxidize sulfur-containing ores, but after consulting the literature, this method becomes less feasible. Brock (1979) has listed the sulfide minerals in order of increasing resistance to oxidation in Table 1, with pyrrhotite being the least difficult mineral to oxidize. Unfortunately, molybdenite ($MoS_2$) is the most difficult to oxidize, following the equation:

$$2MoS_2 + 9O_2 + 6H_2O \rightarrow 2H_2MoO_4 + 4H_2SO_4$$

[Zajic, (1969)].

Table 1

Sulfide Minerals Listed in Order of Increasing Resistance To Oxidation

| | | |
|---|---|---|
| 1. | Pyrrhotite | FeS |
| 2. | Chalchocite | $Cu_2S$ |
| 3. | Covellite | CuS |
| 4. | Tetrahedrite | $3Cu_2S;Sb_2S_3$ |
| 5. | Bornite | $Cu_5FeS_4$ |
| 6. | Galena | PbS |
| 7. | Arsenopyrite | FeAsS |
| 8. | Sphalerite | ZnS |
| 9. | Pyrite | $FeS_2$ |
| 10. | Enargite | $3Cu_2S;As_2S_5$ |
| 11. | Marcasite | $FeS_2$ |
| 12. | Chalcopyrite | $CuFeS_2$ |
| 13. | Molybdenite | $MoS_2$ |

FROM: Brock (1979)

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The oxidation of chalcopyrite ($CuFeS_2$) is also noted in Table 1 as being the second most difficult mineral to oxidize. Thus, the prospects of using bacteria to selectively oxidize the concentrate does not seem very feasible due to its inherent physical structure.

Brierley (1982), and McElroy and Bruynesteyn (1978), have noted that a strain of Sulfolobus briereleyi (a bacteria which grows at 70°C), able to oxidize molybdenite, has the ability to withstand solution concentration up to 750 ppm molybdenum. The subsequent evolution of sulphuric acid. due to the chemical reaction given above, would lower the pH of the concentrate to about 2-3. The current flotation regime of the concentrator plant, however, requires a pH range of around 9-11, which would cause an economic problem if concentrates had to be lowered to pH 3. Thus, the feasibility of:

1. Heating concentrates to 70°C may be uneconomical

2. Lowering the pH of the concentrate would be uneconomical

3. Oxidizing molybdenite may be a time consuming biological process.

Therefore, it seems more reasonable to attack the problem with hydrocarbon-oxidizing microbes, thereby avoiding the problems of oxidizing the molybdenite.

### 3.2.2 Hydrocarbon Degrading Microorganisms

The hydrocarbon-degrading microorganisms encompass the entire range of types of microorganisms including algae, fungi, yeasts, bacteria, and protozoa. The number of genera, however, are restricted to a fairly narrow range, but the species can extend into the hundreds or even thousands. Each of the particular organisms have individual specificities for hydrocarbon degradation. For example, one organism may degrade only methane, another $C_3$ - $C_5$ hydrocarbons, while another only high molecular weight paraffinic substances. In addition, the individual physiology of the particular organism will determine how the hydrocarbon is degraded. It may be oxidized to an alcohol, ketone, or carboxylic acid with no carbon chain degradation. It may also be completely oxidized to carbon dioxide and water by other strains, although this is not always the case. Differing specificities exist depending on the type of hydrocarbon. For our purposes, petroleum hydrocarbons consist of four fractions:

1. Saturated Hydrocarbons (alkanes, branched alkanes, cyclic alkanes, and polycyclic alkanes)

2. Aromatic Hydrocarbons (eg. benzene, toluene, napthalene, etc.)

3. Polar Compounds (alcohols, ketones, aldehydes)

4. Asphaltenes (high molecular weight hydrocarbons, insoluble in most organic solvents).

Ward et al. (1980).
Wyndham and Costerton (1981).

In general terms, these groups represent four individual substrate groups which are degraded in similar ways by individual organisms [Gutnick and Rosenberg (1977)]. Thus, the ability of a microorganism to degrade hydrocarbons can be specifically determined by analyzing the degradation products of the individual fractions of the hydrocarbons. Details for the numerous pathways of hydrocarbon degradation can be found in reviews by Klug and Markovetz (1971), Kallio (1969), McKennon and Kallio (1963), and Van der Linden and Thijsse (1965).

Bacteria

Bacteria are by far the most studied group of microorganisms capable of degrading and/or oxidizing hydrocarbons. Table 2 gives an overall summary of the important bacterial hydrocarbon degraders and their preferential substrates.

Of the petroleum-degrading bacteria, the genus Pseudomonas seems to be the most predominant [Devereux and Sizemore (1982), Walker et al. (1976), Wyndham and Costerton (1981)]. Other important bacteria include Corynebacterium, Mycobacterium, Rhodococcus, Micrococcus, Acinetobacter, and Nocardia. All are facultative hydrocarbon degraders requiring oxygen to metabolize the hydrocarbon.

Table 2.

List of Hydrocarbon Degrading Microorganisms and Their Specificities

| Organism | Degrading | LMW[1] | HMW[2] | Arom[3] | Asphaltenes | Notes | |
|---|---|---|---|---|---|---|---|
| Acinetobacter | | + | + | ?[4] | ? | | Miller & Litsky (1976) |
| Achromobacter | | + | + | ? | ? | | Miller & Litsky (1976) |
| Actinomyces | | + | + | ? | ? | | Davis (1967) |
| Alkaligenes | | + | + | ? | ? | | Miller & Litsky (1976) |
| Arthrobacter | | + | + | ? | ? | degrades carboxylic acid; trehalose lipid surfactant | Feinberg et al. (1980), Miller & Litsky (1976) |
| Bacillus | | + | + | ? | - | | Davis (1967) |
| Beijernickia | | - | - | + | +/-[5] | | Schocken & Gibson (1984) |
| Brevibacterium | | + | + | ? | - | | Davis (1967), Schaeffer et al. (1979) |
| Corynebacterium | | + | + | ? | - | $K^+$, $NH_4^+$ enhances degradation; trehalose lipid surfacant | Van der Linden & Thijsse (1965), Davis (1967), Hasegawa et al. (1980) |
| Flavobacterium | | + | + | ? | + | | Davis (1967), Walker et al. (1976) |
| Methanomonas | | + | +/- | - | - | | Miller & Litsky (1976) |
| Methylobacterium | | + | +/- | + | - | | Patel et al. (1982) |
| Micrococcus | | + | + | + | + | decarboxylates alkanes | Evans (1969), Kallio (1969), Davis (1967) |
| Micromonaspora | | + | + | ? | ? | degrades branched hydrocarbons | Miller & Lisky (1976) |



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| | | | | | | |
|---|---|---|---|---|---|---|
| Mycobacterium | + | + | ? | + | Cu,Zn, Mn toxicity; ketone production; carboxylic acids | Davis (1967), Feinberg et al. (1980) Van der Linder & Thijsse(1965) |
| Nocardia | + | + | +/- | +/- | degrades branched hydrocarbons, trehalose lipid surfactant | Schocken & Gibson (1984) Traxler et al. (1965) |
| Rhodocuccus | + | + | + | ? | | Wyndham & Costerton(1981) |
| Pseudomonas | + | + | + | - | Cu, Ca enhances degradation; produces fatty acids; decarboxylates; rhamnolipid surfactant | Van der Linder & Thijsse (1963), Evans (1969) Solanas & Pares (1984) Miller & Litsky (1976) |
| Streptomyces | + | + | ? | ? | | |

Footnotes:

1. Low Molecular Weight Hydrocarbons, less than $C_{12}$
2. High Molecular Weight Hydrocarbons, greater than $C_{12}$
3. Aromatic Hydrocarbons
4. Unknown
5. Variable Within Genus

## Fungi

The fungi comprise an extremely large variety of hydrocarbon degraders, although their individual attention has been focussed on only a few select species. Table 3 provides a summary of the hydrocarbon degrading fungi. Fungal hydrocarbon degradation mechanisms are similar to that of the bacteria, with a variety of transformation products being produced. The more important hydrocarbon-degrading fungi are: Aspergillus, Cunninghamella, Eupenicillum, Cephalosporium, Fusarium, Helminthosporium, Penicillium, and Spicaria.

## Algae

Only a few species of algae have been noted to degrade hydrocarbons. Most species belong to the genera Chlorella and Prototheca [Miller and Tilsky (1976)]. In order for algae to degrade hydrocarbons, or to grow, a constant supply of light is required. Thus, for a hydrocarbon-degrading algae to be commercially utilized, the substrate must be constantly subjected to light (i.e. in a pond, or via artifical light). Due to the sparse amount of literature on hydrocarbon degrading algae, and their obligate requirement for light, the algae will not be considered for use in this application.

Table 3: Hydrocarbon Degrading Fungi

| Organism | Notes | Reference |
|---|---|---|
| Absidia | - | Miller & Litsky (1976) |
| Acremonium | no growth past $C_{13}$ | Klug & Markovetz (1971) |
| Aspergillus | no degradation past $C_{10}$; branched hydrocarbons | Schaeffer et al. (1979) |
| Botrytis | - | Miller & Litsky (1976) |
| Cephalosporium | good growth | Klug & Markovetz (1971) |
| Chaetomium | - | Miller & Litsky (1976) |
| Chloridium | - | ibid. |
| Cladosporium | - | ibid. |
| Colletotrichum | - | ibid. |
| Cunninghamella | branched hydrocarbons, good growth | Schaeffer et al. (1979) |
| Dematium | - | Miller & Litsky (1976) |
| Epicoccum | - | ibid. |
| Fusarium | good growth, no growth past $C_{13}$ | Klug & Markovetz (1979) |
| Gliocladium | - | Miller & Litsky (1976) |
| Graphium | - | ibid. |
| Helicostylum | - | ibid. |
| Helminthosporium | good growth | Klug & Markovetz (1971) |
| Monila | - | Miller & Litsky (1976) |
| Mucor | - | ibid. |
| Oidiodendron | - | ibid. |
| Paecilomyces | - | ibid. |

| | | |
|---|---|---|
| Pencillium | good growth | Klug & Markovetz (1976) |
| Rhizopus | - | Miller & Litsky (1976) |
| Scolecobasidium | - | ibid. |
| Spicaria | good growth | Klug & Markovetz (1971) |
| Synchephalastrum | - | Miller & Litsky (1976) |
| Trichoderma | - | ibid. |
| Eupenicillum | good growth | Klug & Markovetz (1971) |
| | | Schaeffer et. al. (1979) |

## Protozoa

Protozoans are one-celled or multi-celled colonial organisms which survive by eating benthos, bacteria, or other protozoa. Colin et al. (1984) have studied protozoa, and their bioaccumulation on hydrocarbon substrates. Using the organisms Colpidium and Tetrahymena, it was discovered that the emulsification of oil is slightly increased in the presence of protozoa. The emulsification is dependent on the presence of bacteria, since axenic cultures are unable to induce it. The degradation of oil and paraffins are not strongly influenced by the presence of protozoa when they are simultaneously added with bacteria.

Due to the lack of information on hydrocarbon-degrading protozoa, and the fact that protozoa do not adversely affect hydrocarbon degradation, these organisms will not be used for study.

## Yeasts

Table 4 describes several genera of yeasts reported to utilize hydrocarbons for growth. The more important hydrocarbon-degrading yeasts consist of the genera Candida, Oospora, Rhodotorula, Debaromyces, and Torulopsis. A number of the yeasts also produce surfactants. The surfactants act as detergents and reduce surface tension at the hydrocarbon-water interface allowing the cell to uptake hydrocarbons at an increased rate. The production of surfactants is not limited to yeasts, as bacteria and fungi excrete them. As previously mentioned, the mechanism of hydrocarbon oxidation follows patterns similar to that of the fungi and bacteria.

Table 4

Some Genera of Yeasts Reported to Utilize Hydrocarbons for Growth.

| Organisms | Notes | Reference |
|---|---|---|
| Candida | Produces peptide and fatty acid emulsifiers; degrades past $C_{10}$; prefers straight chains | Gutnick & Rosenberg (1977) Van der Linden & Thijsse (1965) |
| Cryptococcus | - | Klug & Markovetz (1971) |
| Endomyces | - | ibid. |
| Hansenula | - | ibid. |
| Mycotorula | - | ibid. |
| Oospora | excellent growth | Davis (1967) |
| Pichia | - | Klug & Markavetz (1971) |
| Rhodctorula | Sophorose lipid emulsifer; no growth on hydrocarbons below $C_9$ | Wyndham & Costerton(1981) Gutnick & Rosenberg (1977) |
| Torulopsis | surfactant production | Van der Linden & Thijsse (1965) |
| Trichosporon | - | Klug & Markovetz (1971) |
| Saccharomyces | - | ibid. |
| Debaromyces | - | ibid. |
| Torula | - | Davis (1967) |

### 3.2.3 Physical and Chemical Requirements for Growth

All organisms need nutrients to survive. If the growth medium for the organisms are less then optimal, slow growth or no growth will result. The required ingredients for all organisms are the elements C, H, N, O, P, and S. The ions $K^+$, $Cl^-$, $Na^+$, $Mg^{2+}$, and $Fe^{2+}$ are also required. Trace elements are required but their presence does not justify special attention.

#### Oxygen

It is generally accepted that molecular oxygen is required for hydrocarbons to be metabolized by microorganisms [Swain et al. (1978)]. It is essential for metabolism in the hydrocarbon oxidizing bacteria [Babu and Brown (1984)] since the oxygen atom which oxidizes the hydrocarbon comes from $O_2$ and not from water. The enzyme used to catalyze the hydrocarbon oxidation reactions primarily comprise iron containing monooxygenases. Walker et al.(1976) noted that dissolved oxygen concentration directly affects hydrocarbon-oxidizing capacity. Thus, for an effective hydrocarbon-oxidizing system to be established, the aeration of the medium is essential.

Nitrogen

Microorganisms require nitrogen in the form of ammonia ($NH_4^+$) or nitrate ($NO_3^-$). This can be added either artifically in salt form (eg. $NH_4Cl$, $KNO_3$), or a hydrocarbon-utilizing nitrogen-fixing organism can be introduced to supply ammonia. Bacteria of the genera Pseudomonas, Mycobacterium, and Azotobacter are capable of utilizing hydrocarbons for growth, and simultaneously converting atmospheric nitrogen into free ammonia or into cellular substance [Coty, (1967)]. By applying these procedures, cells should be supplied with sufficient quantities of metabolizable nitrogen.

Phosphorous

If phosphorous is not present in the [illegible], it can be cheaply added in the form of phosphate ($PO_4^{3-}$) [Harrison et al. (1980)]. Phosphate is needed for membrane components, protein components, and nucleic acids in cells.

Temperature

Optimum growth temperatures for many organisms are usually in the range of 20° - 40°C (mesophilic bacteria). Growth rates diminish below 20°C, and cells may die above 40°C. For optimum growth, temperature should remain in this range, unless the organisms are suited to higher or lower temperatures.

pH

Optimum pH for many organisms is usually slightly alkaline, around pH 7.4. Some organisms are acidophilic, and grow well at approximately pH 2-4 (i.e. Thiobacillus, Sulfolubus). Some prefer alkaline conditions, but there are relatively few organisms which can survive above pH 8. Thus, if a system for hydrocarbon degradation is to be established with an ore concentrate, the pH adjustment to approximately 7 - 8 would allow for the optimal growth response of bacteria.

### 3.2.4 Products Produced from Hydrocarbon Fermentations

As with any fermentation or bacterial transformation, substrate conversions take place, with the subsequent growth of the microorganisms and the excretion of extracellular products. The two most important microbial products to consider are those from hydrocarbon oxidation and/or degradation, and the production of emulsifying agents, which aid the microorganisms in the uptake of the water insoluble hydrocarbons.

#### Hydrocarbon Transformation

For a complete treatise on hydrocarbon degradation pathways by microorganisms, refer to the following authors: Davis (1967), Evans (1969), Kallio (1969), Miller & Litsky (1976), Schocken & Gibson (1984), Solanas & Pares (1984), Klug & Markovetz (1971), Van der Linder and Thijsse (1965), and Babu and Brown (1984). In general, hydrocarbons are oxidized to either the alcohol, ketone, aldehyde, or carboxylic acid form. Less commonly, epoxidation may occur, [Davis (1967)]. Beta-oxidation, and decarboxylation is common to many hydrocarbon degrading microorganisms [Kallio (1969)].

Hydrocarbons which cannot be used as an energy source, can be oxidized to a more soluble form by a process known as co-oxidation [Miller & Litsky (1976)]. In this process a hydrocarbons can be utilized by the microorganism for growth, and hydrocarbons which cannot be metabolized can be oxidized to alcohol, acid or aldehyde, concomitantly with the growth substrate.

## Surfactant Production

As a consequence of the utilization of hydrocarbon substrates, or in response to such substrates, many organisms produce extracellular metabolites that produce a greater dispersion of hydrocarbon substrate within the aqueous phase [Harrison et al. (1980)]. Such metabolites may be surface-active agents that form micelles, comprising aggregates which surround the hydrocarbon substrates. The non-polar phase within an aqueous solution will hold a greater concentration of hydrocarbon molecules than the aqueous phase itself [Gerson & Zajic (1979)]. The presence of emulsifiers allows for a more rapid uptake of hydrocarbon. Tables 2 and 4 show some surfactant types produced by genera of bacteria and yeasts, respectively.

The production of surfactants may alter the surface properties of the media that the organisms are metabolizing, and thus, may change the surface characteristics of the slurry that is being subjected to flotation. The effect of the production of surfactants from hydrocarbons on a flotation circuit has not been previously detailed. It will be necessary to assess the effects of such surfactant production on the subsequent molybdenum cleaner flotation circuit.

### 3.2.5 Pure Cultures vs Mixed Cultures

Many studies of microorganisms, capable of growing on oil, have dealt with a single microorganism degrading a single hydrocarbon. The effect of single microorganisms on multiple substrates and, conversely, the effect of multiple organisms on a single substrate are poorly understood [Griffin and Cooney (1979)].

Pure cultures have the inherent advantages of degrading and oxidizing substrates in easily defined respiration patterns. Thus, the microbiologist commonly uses pure cultures to study substrate changes accompanied by single species [Solanas and Pares (1984)]. Unfortunately, the reactions are frequently incomplete. When only a few substrates are utilized as an energy source, much residual hydrocarbons is common. Only 30-50 percent of the hydrocarbon was degraded by pure Pseudomonas cultures grown on aromatic petroleum hydrocarbons [Solanas and Pares (1984)].

Mixed population studies show that some compounds which are resistant to degradation by a single microorganism, can be effectively degraded by mixed populations [Griffin and Cooney (1979)]. Mixed cultures harbour many advantages over growth in pure culture, namely:

1. Higher growth rates
2. Stability of cultures
3. Resistance to contamination
4. Nutrient cross-over

   [Harrison (1981)].

Many organisms require growth factors to obtain maximum growth rates. The growth factors may be in the form of vitamins or substrates.

Rambeloarisoa et al. (1984) isolated a mixed population of bacteria grown on crude oil. The bacteria comprised the genera Acinetobacter, Alcaligenes, Flavobacterium, Moraxella, Micrococcus and Bacillus. Eighty one percent of the total hydrocarbons were degraded by the mixed culture. Interestingly, the organisms Flavobacterium, Moraxella, and Bacillus did not degrade hydrocarbons, but their presence was essential for the mixed population to function optimally.

Another mixed culture which shows promise was described by Feinberg et al. (1980). The culture utilizes the microorganisms Mycobacterium rhodochrous and Arthrobacter paraffineus. The mycobacteria degraded dodecylcyclohexane to cyclohexaneacetic acid. The acid was then degraded into smaller units by Arthrobacter. The synergistic degradation of hydrocarbons by mixed species allows for a more complete degradation of the hydrocarbon than degradation by a pure culture.

If a system for the degradation of the hydrocarbon material is to be developed, it would be more suitable to choose a mixed system over a pure culture. The choice of a mixed culture offers many advantages as indicated above, including synergistic degradation.

### 3.2.6 Selection of Microorganisms

#### Through Established Culture Collections

The American Type Culture Collection (ATCC) describes many microorganisms having the ability to degrade hydrocarbons. These strains may have been found in nature or have been commercially used strains utilizing hydrocarbons for the production of chemicals. These strains may be obtained for a small charge, and subsequently, can be tested for the degradation ability on the hydrocarbon-containing substrate. Numerous strains can be tested for hydrocarbon-degrading ability. Subsequently, mixed cultures, which can degrade the hydrocarbon material more completely than pure cultures [Gherna et al. (1985)], will be constructed.

#### From Natural Sources

Microorganisms having the ability to degrade hydrocarbons can be easily obtained from the effluents of petroleum refineries, chemical factories, oil spills, soils, and from surface soils. Stable communities of microorganisms, having been acclimated to their environment, can be isolated. The organisms may harbour enhanced degradation abilities.

Petroleum refinery waste waters contain relatively high concentrations of hydrocarbons. Activated sludge in aerated tanks are used to degrade these hydrocarbons. The activated sludge contains stable communities of fungi, yeasts and protozoa with hydrocarbon degrading ability.

The microbes can be easily obtained and tested for hydrocarbon degrading ability. Wild-type organisms are more stable than laboratory strains that have been acclimated to preset nutrient patterns, or mutated and selected for enhanced product production.

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### 3.2.7 Screening Methods

Hydrocarbon Degradation

For an efficient microbial system to be established, tests must be performed to assess the hydrocarbon degrading capacity of the microbes to the substrate. The degradation should be complete, or as complete as possible, allowing for oxidation of a wide range of substrates, and the co-oxidation of hydrocarbons that cannot be used as substrate.

Fractionation procedures, based on polarity, can separate the hydrocarbon into the maltene, saturate, aromatic, polar, and asphaltene fractions, using organic solvent extraction techniques with column chromatography, and subsequent elution [Wyndham & Costerton (1981), Gutnick and Rosenberg (1977), Rambeloarisoa (1984)]. The extent of degradation on the individual fractions can be assessed in many ways:

1. Gas chromatographic (GC) analysis: Fractions before and after degradation [Solanas and Pares (1984), Fedorak and Westlake (1981)].
2. Gravimetric determination of degradation products: Analytical determination of individual fractions can be performed by comparing the percentage of individual fractions in undegraded samples to degraded samples. Simple extraction methods can be used to obtain this data.
3. Rapid Screening: Kiyohara et al., (1982) have devised a rapid screening procedure for bacteria-degrading water-insoluble hydrocarbons on agar plates. This

method involves spraying of the hydrocarbons on agar plates and examination for clear zones of degradation after suitable incubation times.

Using both analytical screening methods and rapid screening methods, the hydrocarbon-degrading ability of microorganisms will be assessed. The hydrocarbons in the concentrate contain many insoluble hydrocarbons and asphaltenic hydrocarbons. The screening scheme will be centred on the degradation of high molecular weight hydrocarbons.

Toxic Metal Acclimation

Flotation concentrates contain large quantities of heavy metals. Unfortunately, many bacteria find these heavy metals toxic and cannot grow efficiently in their presence. It is necessary to either:

1. Select microorganisms from sources where high concentrations of heavy metals are prevalent (mine drainage, chemical effluents) [Belly and Kydd (1982)].
2. Acclimate strains to heavy metals by selecting those strains having the ability to survive progressively increasing concentrations of heavy metals [Maeda et al. (1983), and Marques et al. (1979)].
3. Introduce extrachromosomal genetic elements into microorganisms which confer heavy metal resistance [Chakrabarty (1978)].

Microorganisms having the ability to tolerate high concentrations of heavy metals will be concomitantly tested for hydrocarbon-degrading/oxidizing ability for selection of both metal tolerant strains and efficient hydrocarbon utilizers.

## 3.3 Experimental Strategy

The results of the literature review have allowed an experimental strategy to be developed for the isolation and enrichment of hydrocarbon degrading microorganisms. The objective is to use organisms to degrade the hydrocarbon species in the contaminated molybdenum concentrate. The experimental plan is detailed as follows:

1. Hydrocarbon degrading microorganisms will be obtained from both existing culture collections and naturally acclimated sources.

2. Microorganisms will be grown on concentrates in aerated flasks to permit maximum oxygen transfer rates. The growth medium will consist of concentrate at low percent solids (10-15%) supplemented with vitamins and inorganic salts.

3. Superior strains will be isolated by subculturing from mixed cultures of microorganisms. This is a process of natural selection whereby superior microorganisms outgrow less efficient species.

4. The selected microorganisms will grow on and degrade the hydrocarbon which is contaminating the concentrates.

5. The effectiveness of separating residual (graphitic) hydrocarbon from treated molybdenite concentrates will be assessed using conventional mineral processing techniques (i.e. flotation, gravity separation).

## 4.0 EXPERIMENTAL INVESTIGATIONS

### 4.1 Materials

#### 4.1.1 Concentrates

Copper - molybdenum rougher concentrates and molybdenum final cleaner concentrates were supplied by the Mine's mill superintendent.

The head assays of the concentrates are listed in Table 5.

#### 4.1.2 Source of Microorganisms

Hydrocarbon degrading microorganisms were obtained from the following sources:

1. The Mine's waters and soils, reported to be adjacent to areas high in hydrocarbon content.
2. Activated sludge microorganisms from the Esso waste petroleum degradation plant at Sarnia, Ontario.
3. Activated sludge microorganisms from the Petro-Canada waste petroleum degradation plant at Clarkson, Ontario.

### 4.2 Methods

#### 4.2.1 Isolation and Enrichment of Hydrocarbon Degraders

Microbial strains, from the original sources, were selected naturally by subculturing them weekly in aerated 3 l flasks at 15% solids of each of the rougher and final ore concentrates. The nutrient formula used is described in Table 6. Parameters including pH, Eh, bacterial numbers and available phosphorous were monitored on a daily basis.

Table 5

CONCENTRATE HEAD ASSAYS

| | %Mo | % C | % Cu | % Fe |
|---|---|---|---|---|
| 1. Copper Molybdenum Concentrate | 2.55 | 2.69 | 24.15 | 7.81 |
| 2. Molybdenum Final Concentrate | 19.22 | 24.32 | 1.13 | 3.93 |
| 3. Copper Concentrate | 1.33 | 1.91 | 25.25 | 20.00 |

Table 6

## BACTERIAL NUTRIENT FORMULATION

| | g/L | g/900L |
|---|---|---|
| $Na_2SO_4$ | 2 | 1800 |
| $Mg\ SO_4$ | 0.2 | 180 |
| $KH_2PO_4$ | 0.5 | 450 |
| $NH_4Cl$ | 1 | 900 |
| $K\ NO_3$ | 2 | 1800 |
| $Fe\ SO_4$ | trace | 0.4 |
| Yeast Extract | 1 | 900 |

Yeast extract was solubilized in a minimal volume of water prior to addition in the reactor. pH was adjusted to 7.5 using NaOH.

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For each of the bacterial sources, 0.1 mL aliquots of 100 fold dilutions were plated on nutrient salts agar and allowed to dry. Solvent extracted hydrocarbon from the molybdenum final concentrate was suspended in a minimal volume of methylene chloride, and sprayed on the plates until a confluent layer of hydrocarbon was deposited on the surface of the plate. After sufficient incubation (about 7 days) at 30°C, individual colonies were selected by observing them under a microscope at low power (100X). Colonies, which showed large zones of clearing ("haloes") of hydrocarbon, were selected. Pure cultures of these bacteria were then propagated on conventional media (plate count agar).

### 4.2.2 Identification of the Principle Bacterial Strains

Isolated microorganisms were originally classified according to conventional bacterial classifications, i.e. Gram reaction, morphology, motility, colony characteristics, pigmentation and presence or absence of spore production.

The API 20E identification strips were subsequently used to further identify and confirm the bacterial strains.

### 4.2.3 Bacterial Degradation of Pure Hydrocarbon Deposited on Silica Sand

Pure hydrocarbon from the final molybdenum concentrate was prepared prior to shake flask studies. Benzene (500 mL) was added to 500 g of ore concentrate in a 2 L beaker. The slurry mixed for 10 minutes then filtered through a Buchner funnel with Whatman #42 filter paper. The residue was returned to the beaker and the mixing and filtering process was repeated twice with 250 mL aliquots of benzene. The solution was evaporated overnight in a fume hood to remove the benzene. Hydrocarbon

was recovered by dissolution in a minimal volume of benzene and evaporation of the remaining benzene in a small glass container. The collected hydrocarbon was then deposited on acid washed silica sand to a level of 5% (w/w). The hydrocarbon was homogeneously coated on the sand particles by adding a minimal amount of benzene, mixing, and allowing the benzene to evaporate in a fume hood. Approximately 250 g of synthetic "tar-sand" was prepared.

A 10 g sample of "tar-sand" was placed in a 250 mL flask and 100 mL of nutrient solution was added. Seven flasks were prepared. Various mixed bacterial cultures were added to each flask. Seven cultures were screened for visible stripping of tar from the sand. The flasks were placed on a reciprocal shaker (Eberbach #5850) for 27 days at 20°C at 120 strokes per minute. Bacterial colony forming units/mL were enumerated on plate count agar daily by the standard plate assay using tenfold dilutions. pH values were monitored daily. Control flasks were also placed on the shaker.

### 4.2.4 Aerated Flask Studies

Bacterial hydrocarbon degradation studies were performed on both copper molybdenum rougher concentrates and final molybdenum concentrates.

For 250 mL flasks, 100 mL of nutrient salts was added to the flask and 15g of concentrate was added to the flask, followed by the appropriate bacterial cultures. Control flasks contained no bacteria, or deionized water and no bacterial culture.

Ore treatment studies in 4000 mL flasks were prepared with the addition of 3000 mL of nutrient solution. For a 15% pulp density, 520 g of concentrate was added to the flask. Agitation was maintained through a four-bladed impellor. Aeration was provided by pumping air from the bottom of the flask through an air stone. The bacterial solutions were subsequently added after pH adjustments.

### 4.2.5 Characterization of Carbonaceous Material

Organic solvents were used to extract the hydrocarbon material from the ore concentrates. Several organic solvents including chloroform, methylene chloride, methanol, heptane and benzene were used. Organic solvents were added to the ore concentrates at 1:1 ratios, mixed, filtered, and the washing step was repeated until no hydrocarbon was detected in the ore concentrate.

The carbonaceous material was concentrated by evaporation of the solvent in a fume hood. Asphaltene hydrocarbons were removed from the hydrocarbon sample by adding 5 mL of heptane for each 1g of hydrocarbon, mixing thoroughly, and filtering through a 0.45 µm millipore filter. Carbon analyses were performed on both rougher and final concentrates before and after organic extraction using a Leco induction furnace and carbon analyzer. Table 7 presents the carbon analyses of the ore samples before and after organic solvent extraction.

Table 7

THE EFFECT OF BENZENE EXTRACTION ON THE ROUGHER AND FINAL MOLYBDENUM ORE CONCENTRATE

| | % C before extraction | % C after extraction | % C extractable | % C non extractable |
|---|---|---|---|---|
| 1. Copper Molybdenum Concentrate | 2.78 | 2.37 | 14.75 | 85.25 |
| 2. Molybdenum Concentrate | 18.09 | 15.08 | 16.64 | 83.36 |

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### 4.2.6 Infrared Spectrophotometric Studies

Infrared spectrophotometric studies were performed on extracted hydrocarbon fractions of bacterially treated and untreated molybdenum ore concentrates. Samples were prepared by adding 10 mL of methylene chloride to every 15g of dried ore concentrate, mixing thoroughly, and filtering through Whatman #42 filter paper. The extracted samples were stored in screw-cap test tubes. Infrared spectrophotometric plots were performed using a Unicam IR1000. Samples were placed in sodium chloride cells with a methylene chloride blank prior to spectrophotometry.

### 4.2.7 Gas Chromatographic Analyses

Gas chromatographic analyses were performed on bacterially treated and untreated "tar-sand" samples. The following shake flask tests were examined by gas chromatography:

1. A flask inoculated with an acclimated mixed bacterial culture in nutrient solution.
2. A negative control in nutrient solution, and
3. An unincubated, unshaken, uninoculated control in deionized water.

The appropriate flasks were placed on a reciprocal shaking machine for 27 days at 20°C and 120 SPM.

Flasks containing tar-sand were allowed to settle for 30 minutes. A primary fluid decantation was performed on each flask of tar-sand and were subsequently washed and decanted twice with 100 mL of deionized water. The samples were washed

with 30 mL of benzene to solubilize the hydrocarbon for removal from the tar sand. After 5 minutes of swirling, the benzene was decanted and saved. The sample was washed again with 30 mL of benzene, mixed, and the solution decanted. The solubilized hydrocarbon fraction was allowed to evaporate under forced air and then weighed.

The extracted hydrocarbon samples were suspended in 10 mL of heptane and mixed well for 30 seconds under vigorous agitation. The asphaltene hydrocarbons were then removed by filtration through a 0.45 μm millipore filter. The filtered heptane soluble fraction was subjected to silica gel column chromatography to separate out the various fractions of hydrocarbon material.

Glass columns (1.1 cm inside diameter x 45 cm) were filled with a slurry of 10 g of 60-200 mesh silica gel in chloroform. When the silica gel had settled, it was drained for 1 minute to facilitate gel packing, leaving a 10 cm head of solvent. A 1 cm layer of acid-washed silica sand followed by a 2 cm layer of activated copper were added to the top of the column. Activated copper was prepared immediately prior to use by soaking powdered copper in concentrated HCl for 2 minutes, rinsing several times with methanol to remove the acid, and then rinsing with chloroform to remove the methanol.

The chloroform was drained until its level reached the top of the copper, and the packed column was washed with 30 mL of heptane. Concentrated samples, in 1.0 mL aliquots, were placed on the column and run into the copper layer where it was held for 2 minutes. The column was then developed, successively with (i) 15 mL heptane, (ii) 13 mL 30% benzene in heptane, and

(iii) 25 mL chloroform. The saturate fraction eluted between the first 20 and 34 mL. Unsaturates and aromatics eluted between 34 and 45 mL. The polar compounds and polyaromatic compounds, and asphaltene compounds eluted between 45 and 55 mL. These samples were collected and concentrated by evaporating the solvent in a fume hood. 2 mL of heptane was subsequently added to dissolve the samples. The samples were then subjected to gas chromatography.

A Hewlett Packard 5830 GC was fitted with a 0.25 mm x 30 m DB-5 column. Helium was used as a carrier gas at a flow rate of 2 mL/min. The injection port and detector were maintained at 400°C. The oven temperature was held at 80°C for 4 minutes, then programmed to increase 8°C/min to 285°C, which was held for 15 minutes.

### 4.2.8 Metallurgical Studies and Preliminary Pilot Scale Degradation

A serially subcultured, naturally acclimated population of activated sludge bacteria were propagated on copper-molybdenum concentrate. The bacterial population was increased stepwise until 3 L of solution at 15% solids contained approximately 3 x $10^6$ bacteria/mL (1 x $10^{10}$ bacteria total). This solution of bacteria was used as inoculum for a large scale treatment of copper-molybdenum concentrate in a total volume of 100 L of solution.

A 200 L drum was cleaned and 100 L of warm tap water ( 35°C) was added to the drum. Nitrogen (as $NH_4NO_3$) and phosphorous (as $KH_2PO_4$) were added until the concentrations reached 2.5 mM and 1.24 mM, respectively. The 3 L of seed material was added to the reactor contents, followed by 10 kg of copper-molybdenum concentrate.

Agitation was maintained at a constant rate fast enough to allow adequate mixing of the reactor contents and an even distribution of dissolved oxygen.

Aeration was provided by either pure oxygen at a rate of 2 L/minute or from an air compressor at a rate of approximately 10 L/minute.

Temperature was maintained at 30°C using heating strips which contacted the exterior of the drum and insulation was provided with pink fiberglass insulation adjacent to the heating strips. Solution samples were taken daily and monitored for pH, Eh and bacterial numbers. Samples in 100 mL volumes were saved for subsequent infrared spectrophotometric analyses.

Following completion of the pilot scale bacterial degradation of the copper-molybdenum rougher concentrate, the slurry was allowed to settle, the remaining solution decanted, the solids filtered and dried at room temperature. The remaining concentrate was used for flotation and vanning test work.

Flotation test work was performed using the Denver D-12 flotation machine at 1500 RPM using a stainless steel cell containing 750 g of solid in 5500 mL of water (12% P.D.). Two flotation schemes were used:

I. molybdenum cleaning as performed in the Mine's circuit at pH 8.5, using sodium cyanide at 1.5 lb/ton

II. carbon flotation using the reagent Orzan A to effectively depress the molybdenum. Orzan was used at 20 lb/ton at pH 10.6.

The pulp was conditioned for 5 minutes and floated for 3 minutes for each test.

A molybdenum cleaner concentrate was floated from the treated copper-molybdenum concentrate depressing the copper minerals, followed by a secondary Orzan A flotation to float hydrocarbon and depress molybdenite.

Bartles-Mozley vanning tests were performed on both degraded and undegraded copper molybdenum concentrates. Slurries of approximately 1000 g samples at 20% P.D. were applied at each vanning test. The vanning table was previously set up to optimize the separation of hydrocarbon from molybdenite.

### 4.2.9 Mineralogical Examinations

Mineralogical examinations were performed on the the bacterially treated and untreated molybdenum ore concentrates.

Polished sections were prepared from the bacterially treated and untreated concentrates by impregnating the dried samples in epoxy. The samples were mounted on glass slides and scanned at magnifications of 200X and 500X. Grains of specific interest were photomicrographed using a 35 mm camera attached to the Leitz Laborlux 12 polarizing microscope equipped with a reflected light source.

Mineral identifications were based on the qualitative optical properties using the petrographic microscope.

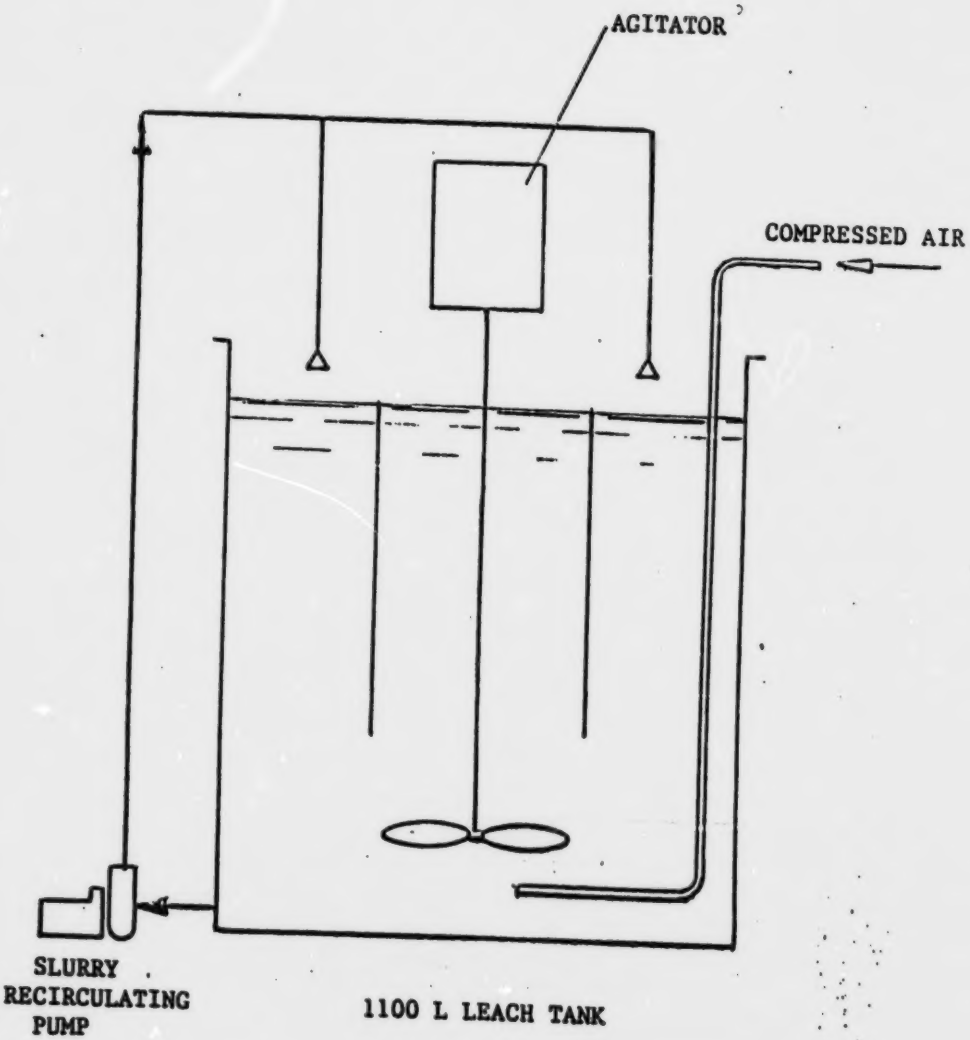
### 4.2.10 Pilot Scale Treatment of Molybdenum Concentrate

A pilot scale batch bacterial treatment was performed on 78 kg of blended molybdenum ore concentrate. The head assay for the concentrate is illustrated in Table 5. A 10 L bacterial seed culture, acclimated to the blended ore concentrate, was prepared as inoculum for the pilot scale test. The seed culture was prepared by growing acclimated bacteria in 3 L batches on the concentrate at 8% solids in nutrient medium for five days, filtering the solids, and saving the remaining bacterial solution. This process was continued until 10 L of solution was grown.

A pilot scale reactor was constructed to treat the ore concentrate. Figure 1 illustrates the reactor and its components. Warm tap water was added to the reactor until 900 litres was reached. The 78 kg of ore concentrate was then slowly added to the reactor with the agitator running to ensure proper wetting. The nutrient formula was then added. Its formulation is shown in Table 6. Sodium hydroxide was used to raise the pH to 7.5. Finally, 10 L of the bacterial solution was added to the reactor to start the ore treatment process. The bacterial population at the start of the treatment was $4.1 \times 10^5$ colony forming units (CFU)/mL.

Aeration to the reactor was provided through an air compressor to provide oxygen to the system. Agitation was maintained to provide adequate mixing of the reactor contents and to aid in the dispersion of air bubbles. A novel apparatus had to be set up on the reactor to control the froth due to the natural flotation of the minerals (See Figure 1).

FIGURE 1. REACTOR SYSTEM EMPLOYED FOR PILOT SCALE TREATMENT OF 78 kg OF MOLYBDENUM ORE CONCENTRATE.



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The slurry recirculating pump was set up to kill the froth by pumping two spray jets of slurry on top of the reactor solution.

Bacterial numbers, pH, temperature, Eh readings and daily samples were taken to follow the ore treatment process. Following ten days of treatment, the solids were allowed to settle, three quarters of the bacterial solution discarded, and the solution replaced with fresh nutrient. This was performed to allow bacteria to remain in the logarithmic phase of growth. Solution replacement was performed on a 48 hour basis until the treatment was terminated on day 15.

When settling the solids to decant the bacterial solution, it was noted that approximately 2% of the solids in the reactor did not settle after 24 hours. Settling occurred in the uninoculated control flasks, and was tentatively attributed to the formation of a bacterial extracellular polysaccharide or surfactant, causing the continuous suspension of ore particles.

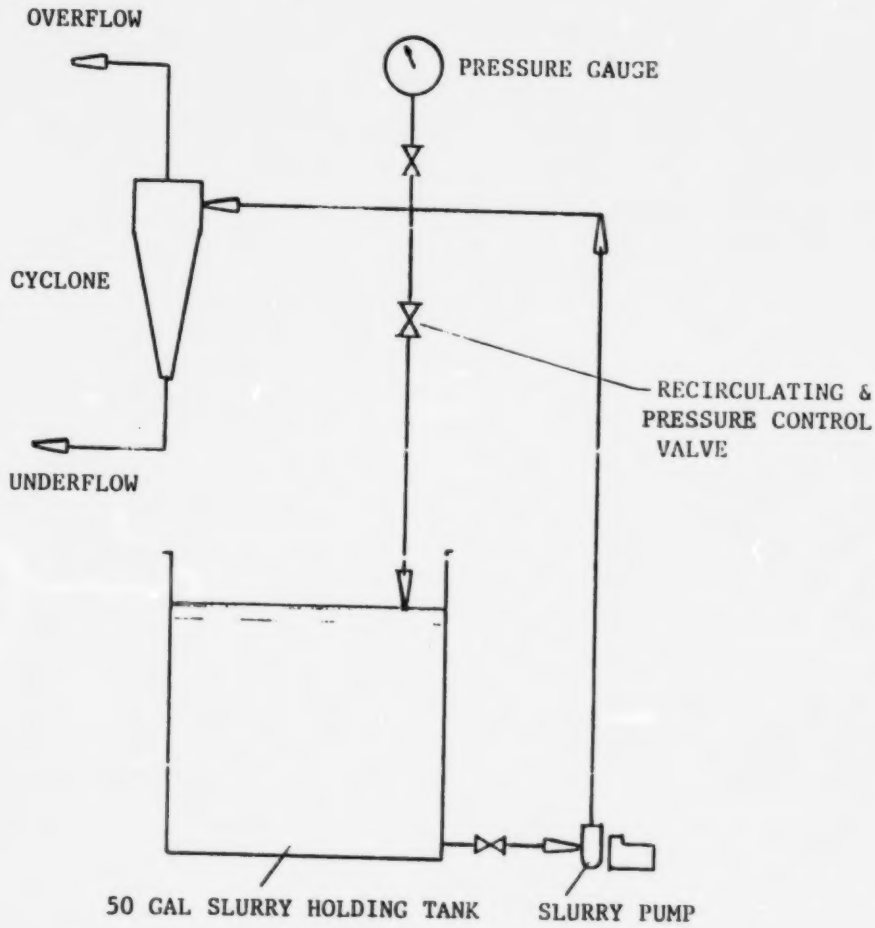
At the end of the treatment schedule 15 days after start-up, the solids were settled and the reactor contents were prepared for subsequent cycloning studies.

### 4.2.11 Cyclone Testing of Treated Molybdenum Concentrate

A one stage cyclone set-up was constructed to separate carbon from the treated molybdenum concentrate. Figure 2 illustrates the cyclone set-up.

Following bacterial treatment of the ore concentrate, the agitator and aerator from the treatment vessel were turned off,

Figure 2 SINGLE STAGE CYCLONE SETUP FOR TESTING METAL RECOVERIES FROM A BACTERIALLY TREATED MOLYBDENUM ORE CONCENTRATE



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the solids were allowed to settle, and 500 L of supernatant was transferred into holding vessels. After 24 hours of settling, the supernatant was still dark grey and opaque. To settle the suspended solids, lime was added until the remaining particles flocculated.

Cycloning studies were begun on the decanted slurry. Slurry densities of 3, 7.3, 9.3 and 12% solids were set up in advance of each cyclone run. Cyclone pressures were adjusted to 25, 30, 35 or 40 PSI for each run by adjusting the backflow valve until the desired pressures were reached.

Timed runs for 20 or 30 seconds were performed on the prepared slurries at the different pump pressures. After weighing, filtering and drying, assays were performed for carbon, Mo, Cu, and Fe.

## 5.0 RESULTS

### 5.1 Identification of the Principle Bacterial Strains

At the conclusion of the shake flask experiments, bacterial species from the most promising cultures were identified.

The organism Pseudomonas sp. was the most predominant bacterial species. The organisms Arthrobacter sp., Mycobacterium sp. and Flavobacterium sp. were present in the culture in slightly lower populations than Pseudomonas sp. The organisms Bacillus sp. and Micrococcus sp. were identified in original enrichments but did not survive serial subculturing.

### 5.2 Characterization of Carbonaceous Material

Table 5 presents the average head assays for the copper molybdenum rougher concentrate and final molybdenum concentrate for % Mo, C, Cu and Fe content.

The organic extraction of hydrocarbon gave the best results when benzene was used as the solvent. Methylene chloride and chloroform ranked next in efficiency to extract hydrocarbon from the molybdenum ore concentrates.

The benzene extracted material was found to occur in two forms:

1. An "asphalt-like" substance, very viscous and sticky, completely insoluble in water.

2. A solid, extractable hydrocarbon which can be precipitated from the benzene-extracted asphalt fraction. By redissolving the hydrocarbon in heptane and filtering the

mixture through a 0.45 μm millipore filter, the solid fraction formed a residue on the filter.

A carbon analysis was performed on both rougher and final ore concentrates before and after benzene extraction. Table 7 summarizes the percentage loss of carbon in these samples. Approximately 15% of the hydrocarbon in the rougher and final ore concentrates was extractable.

## 5.3 Infrared Spectrophotometric Studies of Treated and Untreated Concentrates

Infrared spectrophotometry was performed on bacterially treated and untreated samples to determine the effects of the bacterial treatment on the hydrocarbon as compared to undegraded control samples. Figures 3 and 4 show infrared plots of the undegraded and degraded samples, respectively. The significant difference in the two graphs was the appearance of the predominant O-H peak at 3400 $cm^{-1}$. This was a strong indication of the presence of alcohol moieties resulting from microbial metabolism. No O-H bonding could be found in the undegraded sample (Figure 3). The remaining peaks are representative of aliphatic hydrocarbons (i.e. carbon-hydrogen and carbon-carbon bonding), with some indications of sulfur (at 2550 $cm^{-1}$).

Figure 3

INFRARED SPECTROPHOTOMETRIC (IR) PLOT OF AN UNTREATED COPPER-MOLYBDENUM CONCENTRATE HYDROCARBON EXTRACT (SOLVENT: $CH_2Cl_2$ NaCl CELL)

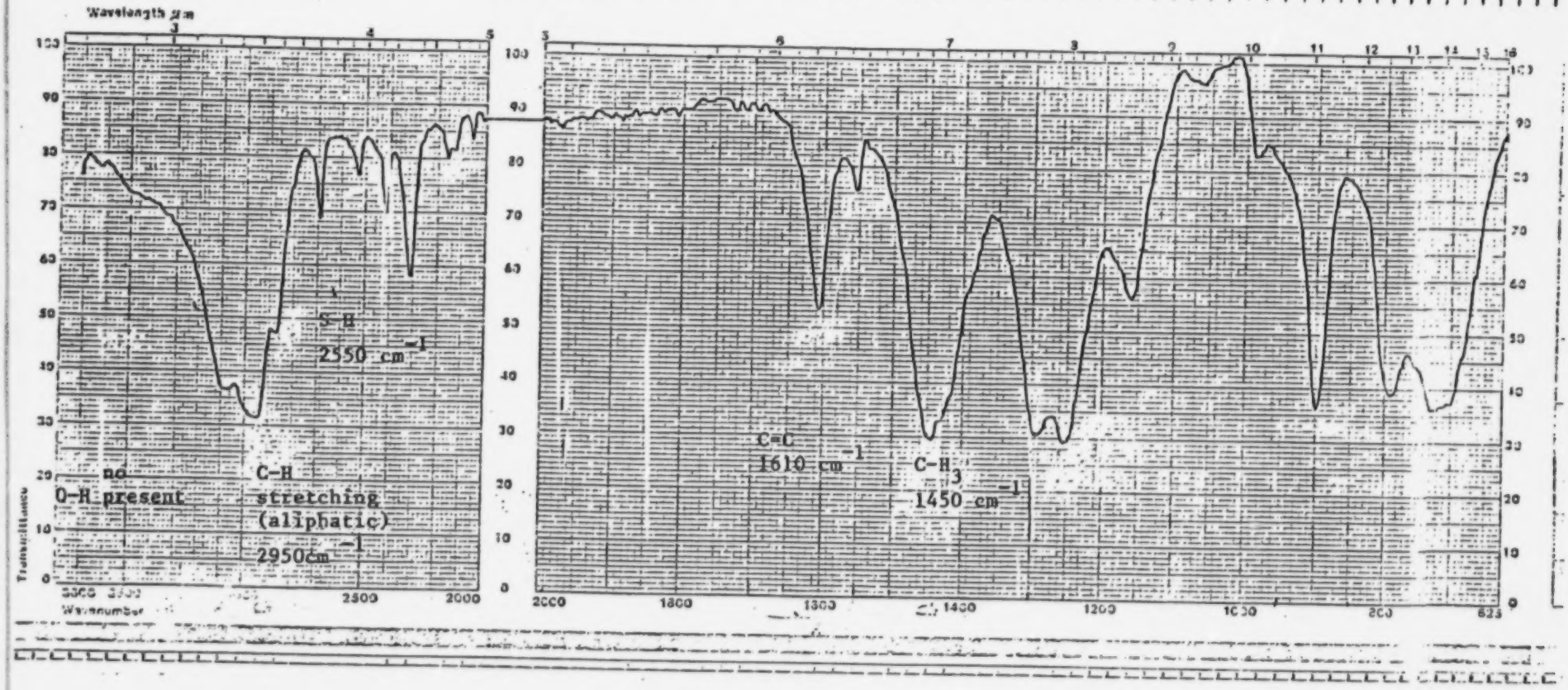
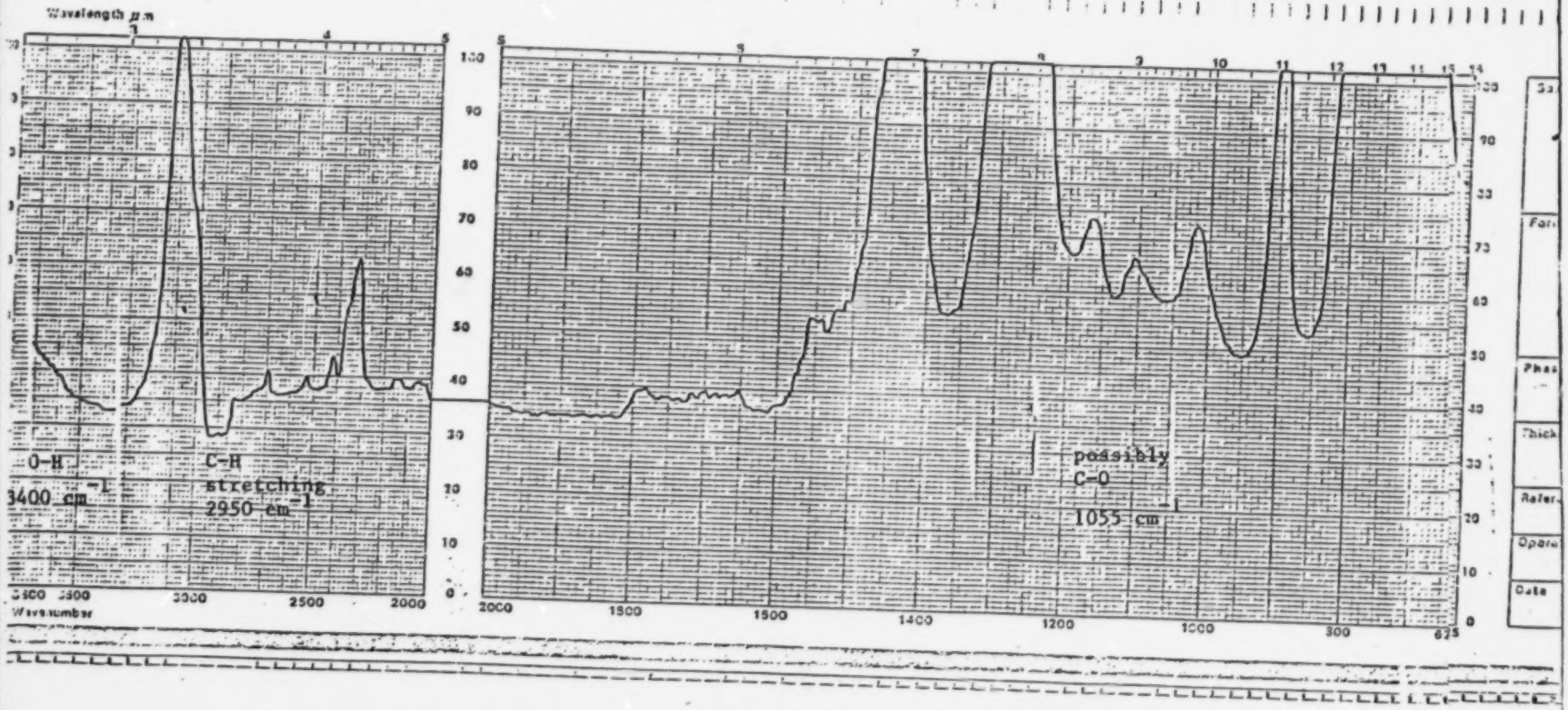


Figure 4

INFRARED SPECTROPHOTOMETRIC PLOT (IR) OF A BACTERIALLY TREATED COPPER-MOLYBDENUM CONCENTRATE HYDROCARBON EXTRACT (SOLVENT: $CH_2Cl_2$ NaCl CELL)



## 5.4 Gas Chromatographic Assessment of Hydrocarbon Fractions Present in Bacterially Treated and Untreated Synthetic "Tar Sand" Samples

The appropriate flasks were placed on a reciprocal shaking machine for 27 days. The hydrocarbon was removed from the "tar-sand" by repeatedly washing with benzene. Concentrated fractions of asphaltene-free hydrocarbon were subjected to column chromatography to separate the hydrocarbons into the saturate, aromatic and polar fractions. These fractions were concentrated and subjected to gas chromatography. The chromatograms from the gas chromatographic analyses are shown in Appendix II .

The gas chromatograms from the hydrocarbon fractions showed impurities from both solvents and from plasticisers. However, noticable trends and hydrocarbon bands could be resolved from the chromatograms. A basic summary concerning the chromatograms was interpreted as follows:

The unshaken negative control showed the majority of saturated hydrocarbons in the $C_{19}$ to $C_{30}$ range, with the majority of aromatic and saturated hydrocarbons concentrating between $C_{17}$ and $C_{25}$. Some aromatics and polars less than $C_{11}$ were noticable.

The shaken control in nutrient solution showed some decomposition of the high molecular weight hydrocarbons, where less high molecular weight hydrocarbons were observed around $C_{25}$. Distribution of peaks in the chromatogram have similarly shifted to the left in relation to the unshaken control.

The bacterially-treated hydrocarbon profiles showed distinct differences from the control sample chromatograms. The saturate fraction showed very few hydrocarbons other than the solvent peaks, indicating that the bacteria have completely consumed the saturated hydrocarbons from the tar-sand. Accordingly, less high molecular weight aromatic compounds were observed on the chromatograms of the treated tar sand. As a result of the bacterial treatment, low molecular weight polar compounds were found at less than $C_{11}$ indicating the degradation of high molecular weight aromatic and saturate hydrocarbons into low molecular weight polar hydrocarbons.

Results obtained from these chromatograms were not conclusive. Solvent impurities and contaminants hampered degradation analysis and complicated the GC plots.

## 5.5 Assessment of Bacterial Growth on Extracted Hydrocarbon Deposited on Silica Sand

Seven mixed bacterial cultures were screened for visible stripping of tar from the tar sand. The three most successful cultures were selected for bacterial identification and further studies. Table 8 shows the evolution of bacteria as colony forming units/mL and the change in pH over time. Bacterial populations commonly peaked at the third or fourth day of growth and gradually levelled to a constant population. The nutrient medium was highly buffered to maintain neutral pH. The pH fluctuated but eventually stabilized at slightly acid levels. Natural oxidation of hydrocarbons in the control flask containing only water demonstrated a decrease in the pH to 2.8 in the absence of the buffer.

Table 8

pH VALUES AND BACTERIAL NUMBERS (as colony forming units/mL) FROM SELECTED BACTERIAL CULTURES GROWN ON EXTRACTED "TAR SAND" AND COPPER-MOLYBDENUM CONCENTRATE

| Flask # | | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 27 |
|---|---|---|---|---|---|---|---|---|
| 1. Tar Sand | pH: | 7.31 | 7.64 | 7.68 | 7.70 | 7.77 | 7.71 | 6.67 |
| with 4 strains | CFU/mL: | $4.5\times10^{7}$ | $1.2\times10^{10}$ | $2.8\times10^{10}$ | $3\times10^{10}$ | $4.2\times10^{8}$ | $3\times10^{8}$ | $3\times10^{8}$ |
| 2. Tar Sand | pH: | 7.28 | 7.56 | 7.60 | 7.47 | 7.43 | 7.38 | 6.32 |
| with 4 strains | CFU/mL: | $9.3\times10^{6}$ | $4\times10^{10}$ | $4.7\times10^{8}$ | $7.4\times10^{8}$ | $8\times10^{8}$ | $7.3\times10^{8}$ | $7\times10^{8}$ |
| 3. Tar Sand | pH: | 7.38 | 7.48 | 7.63 | 7.65 | 7.61 | 7.55 | 7.06 |
| with undefined mixed cutlure | CFU/mL: | $6.6\times10^{6}$ | $4\times10^{10}$ | $4.6\times10^{8}$ | $4\times10^{8}$ | $1\times10^{7}$ | $2\times10^{8}$ | $2\times10^{8}$ |
| 4. Culture from | pH: | 6.68 | 6.52 | 6.69 | 6.58 | 6.59 | 6.56 | 6.62 |
| #1 on Cu-Mo Conc. | CFU/mL: | $2.1\times10^{5}$ | $3.4\times10^{8}$ | $6.8\times10^{8}$ | $1.4\times10^{8}$ | $2\times10^{8}$ | $3\times10^{7}$ | $1\times10^{7}$ |
| 5. Culture from | pH: | 6.69 | 6.60 | 6.78 | 6.62 | 6.65 | 6.60 | 6.68 |
| #2 on Cu-Mo Conc. | CFU/mL: | $2.9\times10^{5}$ | $5\times10^{8}$ | $1.4\times10^{10}$ | $5.2\times10^{8}$ | $4\times10^{8}$ | $3.2\times10^{8}$ | $2\times10^{8}$ |
| 6. Culture from | pH: | 6.72 | 6.37 | 6.54 | 6.48 | 6.50 | 6.54 | 6.32 |
| #3 on Cu-Mo Conc. | CFU/mL: | $7.1\times10^{4}$ | $1.0\times10^{8}$ | $1.4\times10^{8}$ | $2\times10^{8}$ | $1\times10^{8}$ | $3\times10^{7}$ | $8\times10^{7}$ |
| 7. Control | pH: | 7.50 | 7.64 | 7.35 | 7.48 | 7.53 | 7.68 | 5.91 |
| Tar Sand + mineral Salts | | | | | | | | |
| 8. Control | pH: | 6.62 | 6.37 | 7.43 | 7.59 | 7.41 | 7.35 | 2.81 |
| Tar Sand + water | | | | | | | | |
| 9. Control | pH: | 6.70 | 6.25 | 6.12 | 5.97 | 6.05 | 6.00 | 5.82 |
| Cu-Mo Conc. + Mineral Salts | | | | | | | | |

Figure 5 displays a photograph of an unshaken sample of tar-sand in water. Free sand particles were not observed. Figure 6 shows a photograph of an uninoculated tar sand sample suspended in mineral salts solution and shaken on a reciprocal shaker for 8 days. Some sand particles have been freed due to mechanical shaking, but the majority of the sand particles were in contact with the hydrocarbon material. Figure 7 shows a photograph of a bacterially inoculated flask in mineral salts solution shaken for 8 days. A comparison of Figure 7 and Figure 6, of the bacterially inoculated flask shows a definite enhancement of the separation of tar from sand particles. However, some sand remained in contact with the tar. Similarly, Figure 8 displays a bacterially-treated flask. The silica sand exhibited increased separation from the tar in the treated flask than the control flask. When the solutions from the bacterially-treated flasks were examined, stringy pieces of tar-like material were observed floating in the nutrient solution. This demonstrates the partial emulsification of the hydrocarbon, rendering it less "sticky" than the original hydrocarbon.

Close-up photographs of the tar-sand can be seen for both of the control flasks (Figures 9 and 10), and the bacterially inoculated flasks (Figures 11 to 13). Very few free sand particles can be seen in the shaken control tar-sand sample (Figure 9).

Figure 10 is an unshaken control tar-sand and shows even fewer free sand particles. The bacterially-treated tar-sands shown in Figures 11 to 13 illustrate that the sand particles have been liberated from the hydrocarbon to a substantially greater extent than the uninoculated controls.

Figure 5
Unshaken, Undegraded tar-sand mixture
(Control) in water

Figure 6
Uninoculated, shaken tar-sand mixture in mineral
salts solution (Control) - 8 days shaking

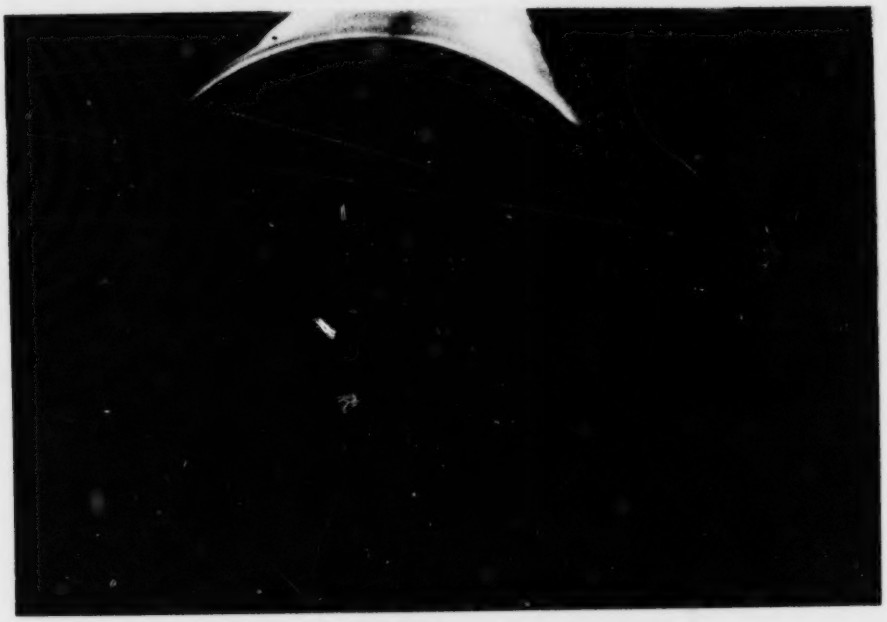


Figure 7
Inoculated tar-sand mixture in minerals salts solution. Flask #1 - shaken 8 days. Notice how mixture is "free flowing" and not agglomerated together.

Figure 8
Inoculated tar-sand mixture in minerals salts solution. Flask #3 - shaken 8 days. Notice free grains of silica present.



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Figure 9
Uninoculated tar-sand sample. Flask #8 -
shaken 8 days. Still sticky and ashphalt like.

Figure 10
Uninoculated tar-sand sample - no shaking.

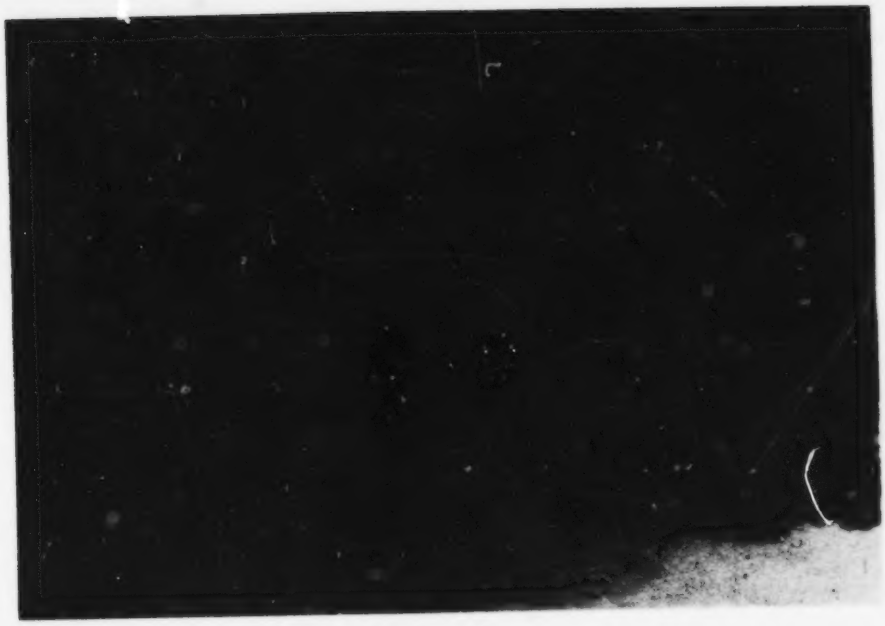

Figure 11
Inoculated tar-sand sample. Flask #1 - shaken 8 days. Notice grains of silica where all hydrocarbon has been degraded.

Figure 12
Inoculated tar-sand mixture. Flask #2 - shaken 8 days. Notice grains of free silica where all hydrocarbon has been degraded.



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Figure 13
Inoculated tar-sand mixuture. Flask #3 -
shaken 8 days

Table 9

RESIDUAL HYDROCARBON DETERMINATION FROM DEGRADED AND UNDEGRADED "TAR SAND" RESIDUES.

| Flask # | Initial Weight of Hydrocarbon | Wt. Hydrocarbon after (g) | Wt lost(%) |
|---|---|---|---|
| 1. Inoculated | 0 .50 | 0.4468 | 10.64 |
| 2. Inoculated | 0 .50 | 0.3822 | 23.56 |
| 3. Inoculated | 0 .50 | 0.3533 | 29.34 |
| 4. Control | 0 .50 | 0.4763 | 4.74 |

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Following completion of the experiment, the hydrocarbon from each of the flasks was thoroughly washed with water and stripped from the sand by washing the sand with benzene. The benzene was removed by evaporation and the residual hydrocarbon weighed. Table 9 shows the percentage of hydrocarbon lost due to the bacterial treatment compared to the control. A considerably greater amount of hydrocarbon was lost through the bacterial treatment as compared to the uninoculated control.

## 5.6 Metallurgical Evaluation of Pilot Degraded Concentrates

During the molybdenum cleaning it was evident that the carbon was floating with the molybdenum, but the froth was much more controllable than was experienced on the undegraded material. The concentrate appeared less slimy and it was anticipated that the froth characteristics made the concentrate amenable to further cleaning.

Primary Cleaning of Degraded Copper-Molybdenum Rougher Concentrate (one stage of cleaning)

(only NaCN added)

| | % Distribution | | |
|---|---|---|---|
| | Wt | C | Mo |
| Conc. | 60.32 | 92.86 | 50.43 |
| tail | 39.68 | 7.32 | 49.57 |
| | 100.00 | 100.00 | 100.00 |

Calc Head 3.25% C 4.45% Mo

The Orzan was ineffective for depression of the molybdenum and did not float the carbon from the final molybdenum concentrate (see tables below).

Secondary Flotation of Bacterially Degraded Concentrate Treated with Reagent Orzan A at pH 10.6

| | % Distribution | | |
|---|---|---|---|
| | Wt | C | Mo |
| Conc. | 42.54 | 58.30 | 39.78 |
| tail | 57.46 | 41.70 | 60.22 |
| | 100.00 | 100.00 | 100.00 |

Calc Head 12.01% C 4.09% Mo

Flotation of Final Molybdenum Concentrate with Reagent Orzan A at pH 10.6

| | % Distribution | | |
|---|---|---|---|
| | Wt | C | Mo |
| Conc. | 6.80 | 9.25 | 1.94 |
| tail | 93.20 | 90.75 | 98.06 |
| | 100.00 | 100.00 | 100.00 |

Calc Head 3.25% C 4.45% Mo

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Bartles-Mozley vanning tests were performed on both degraded and undegraded copper-molybdenum concentrates. Attempts were made to detect an increase in separation efficiency due to the bacterial treatment as compared to the untreated control.
The shaking table tests, however, did not permit an effective separation of carbon from molybdenite, with a large percentage of carbon reporting to the concentrate in both cases. In comparing the response of the concentrate on the shaking table, the degraded material seemed to flow better and distinct particles of carbon and molybdenite could be separated. With the untreated concentrate, the molybdenite and carbon agglomerated making operation of the vanner difficult.

## 5.7 Pilot Scale Treatment of Molybdenum Concentrate

The data obtained from the pilot run is illustrated in Table 10. Bacterial numbers in the reactor remained relatively constant throughout the treatment. Bacterial counts around $10^8$ colony forming units per mL were common values. pH values remained relatively constant, within the pH range 7.18 - 7.67. The products of hydrocarbon degradation were not known, but the results indicate that organic acid production was insufficient to drastically lower the pH value. Temperature values in the reactor increased from 19.5°C to 29.3°C throughout the 8-day oxidation process.

Table 10

BACTERIAL NUMBERS (as colony forming units/mL), pH, TEMPERATURE AND Eh MEASUREMENTS FROM A PILOT SCALF BACTERIAL TREATMENT OF 78 kg OF BLENDED MOLYBDENUM CONCENTRATE AT 8% SOLIDS

| Day | CFU/mL | pH | Temp( C) | Eh(mV) | Notes |
|---|---|---|---|---|---|
| 0 | $4.1 \times 10^5$ | 7.49 | 19.5 | +28 | |
| 1 | $6.9 \times 10^7$ | 7.40 | 22.0 | +66 | |
| 2 | $8.2 \times 10^7$ | 7.54 | 24.0 | +54 | |
| 3 | $8.9 \times 10^7$ | 7.62 | 25.0 | +42 | |
| 6 | $1.0 \times 10^8$ | 7.53 | 25.0 | +148 | |
| 7 | $6.2 \times 10^7$ | 7.67 | 26.5 | [illegible] | |
| 8 | $5.2 \times 10^7$ | 7.43 | 29.5 | [illegible] | aeration off |
| 9 | $1.5 \times 10^8$ | 7.18 | 29.5 | +57 | |
| 10 | $5.4 \times 10^8$ | 7.48 | 29.5 | +125 | solution replacement |
| 13 | $6.4 \times 10^8$ | 7.30 | 25.0 | +95 | solution replacement |
| 14 | $6.2 \times 10^7$ | 7.18 | 25.5 | +125 | |
| 15 | $3.5 \times 10^8$ | 7.21 | 24.5 | +120 | end of treatment |

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The temperature increase was most probably due to heat generated by bacterial metabolism. Accordingly, Eh values rose from relatively low oxidizing environments (+28 mV) to higher oxidizing potentials (+180 mV) over the seven day period.

## 5.8 Cyclone Testing of Treated Molybdenum Concentrate

The Mine's most successful in plant method to date of separating hydrocarbon from molybdenum is the use of one-inch diameter cyclones between the fourth and fifth molybdenum cleaner concentrates. Figure 2 illustrates the cyclone equipment used to treat the molybdenum ore concentrate at Witteck.

The data for the cyclone runs is presented in Table 11. The observed trend from the data is two-fold:
1. Carbon rejections increased as slurry density is increased, and 2. Molybdenum recovery decreased as slurry density increased, but can be compensated for by increasing pump pressure.

Figure 14a illustrates carbon rejections obtained from the cyclone runs and those obtained from the Mine's previous runs. Figure 14a indicates that carbon rejections were considerably higher in the bacterially treated concentrate. Molybdenum recoveries were less efficient than the results obtained from the Mine. Figure 14b shows the molydbenum recoveries obtained from the cyclone runs and those obtained from the mine's previous runs. It is anticipated that further cycloning of optimally treated concentrates will give greater molybdenum recoveries.

Table 11

CYCLONING RESULTS FROM A BACTERIALLY TREATED BLENDED MOLYBDENUM CONCENTRATE

I : Density = 3% Solids pH 7.5

| | gm wt | % wt | Assays C | Mo | Cu | Fe | % Distribution C | Mo | Cu | Fe |
|---|---|---|---|---|---|---|---|---|---|---|
| 25 PSI | | | | | | | | | | |
| Overflow | 104.0 | 37.72 | 33.8 | 6.23 | 7.70 | 9.37 | 80.1 | 18.3 | 38.4 | 33.2 |
| Underflow | 171.7 | 62.28 | 5.1 | 16.80 | 7.49 | 10.20 | 19.9 | 81.7 | 61.6 | 66.8 |
| Calc. Head | 275.7 | 100.00 | 15.97 | 12.81 | 7.57 | 9.31 | 100.0 | 100.0 | 100.0 | 100.0 |
| 30 PSI | | | | | | | | | | |
| Overflow | 60.3 | 38.24 | 34.6 | 5.55 | 7.76 | 8.36 | 76.6 | 18.3 | 39.2 | 34.0 |
| Underflow | 97.4 | 61.76 | 6.53 | 15.34 | 7.45 | 10.03 | 23.4 | 81.7 | 60.8 | 66.0 |
| Calc. Head | 157.7 | 100.00 | 17.26 | 11.60 | 7.57 | 9.39 | 100.0 | 100.0 | 100.0 | 100.0 |
| 40 PSI | | | | | | | | | | |
| Overflow | 94.7 | 37.24 | 35.0 | 5.89 | 7.78 | 8.50 | 76.6 | 18.7 | 37.8 | 33.2 |
| Underflow | 159.6 | 62.76 | 6.35 | 15.17 | 7.61 | 10.14 | 23.4 | 81.3 | 62.2 | 66.8 |
| Calc. Head | 254.3 | 100.00 | 17.02 | 11.71 | 7.67 | 9.53 | 100.0 | 100.0 | 100.0 | 100.0 |

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Table 11
Cont.d

II Density : 7.3 % Solids pH = 9.5

| | gm wt | % wt | Assays C | Mo | Cu | Fe | % Distribution C | Mo | Cu | Fe |
|---|---|---|---|---|---|---|---|---|---|---|
| 25 PSI | | | | | | | | | | |
| Overflow | 268 | 47.95 | 33.6 | 5.85 | 7.46 | 8.13 | 87.18 | 25.20 | 47.88 | 42.39 |
| Underflow | 291 | 52.05 | 4.55 | 16.0 | 7.48 | 10.18 | 12.82 | 74.80 | 52.12 | 57.61 |
| Calc. Head | 559 | 100.00 | 18.48 | 11.13 | 7.47 | 9.20 | 100.00 | 100.00 | 100.00 | 100.00 |
| 30 PSI | | | | | | | | | | |
| Overflow | 271 | 47.46 | 34.65 | 6.57 | 7.78 | 8.62 | 87.67 | 25.88 | 48.78 | 43.53 |
| Underflow | 300 | 52.54 | 4.40 | 17.0 | 7.38 | 10.10 | 12.33 | 74.12 | 51.22 | 56.47 |
| Calc. Head | 571 | 100.00 | 18.76 | 12.05 | 7.57 | 9.40 | 100.00 | 100.00 | 100.00 | 100.00 |
| 35 PSI | | | | | | | | | | |
| Overflow | 284 | 47.18 | 34.2 | 6.06 | 7.46 | 8.28 | 86.59 | 23.83 | 46.62 | 41.58 |
| Underflow | 318 | 52.82 | 4.73 | 17.30 | 7.63 | 10.39 | 13.41 | 76.17 | 53.38 | 58.42 |
| Calc. Head | 602 | 100.00 | 18.63 | 12.00 | 7.55 | 9.37 | 100.00 | 100.00 | 100.00 | 100.00 |
| 40 PSI | | | | | | | | | | |
| Overflow | 285 | 46.12 | 35.85 | 5.8 | 7.18 | 8.00 | 86.72 | 22.50 | 45.17 | 40.50 |
| Underflow | 333 | 53.88 | 4.70 | 17.1 | 7.46 | 10.06 | 13.28 | 77.50 | 54.83 | 59.50 |
| Calc. Head | 618 | 100.00 | 19.07 | 11.89 | 7.33 | 9.11 | 100.00 | 100.00 | 100.00 | 100.00 |

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Table 11
Cont.d

III Density : 9.3 % Solids pH = 9.5

| | gm wt | % wt | Assays C | Mo | Cu | Fe | % Distribution C | Mo | Cu | Fe |
|---|---|---|---|---|---|---|---|---|---|---|
| 25 PSI | | | | | | | | | | |
| Overflow | 331 | 49.70 | 34.0 | 6.35 | 8.56 | 8.75 | 89.24 | 28.51 | 50.44 | 44.35 |
| Underflow | 335 | 50.30 | 4.05 | 15.73 | 8.31 | 10.85 | 10.76 | 71.49 | 49.56 | 55.65 |
| Calc. Head | 666 | 100.00 | 18.94 | 11.07 | 8.43 | 9.81 | 100.00 | 100.00 | 100.00 | 100.00 |
| 30 PSI | | | | | | | | | | |
| Overflow | 337 | 49.41 | 33.5 | 5.50 | 7.94 | 8.11 | 88.99 | 23.03 | 51.31 | 45.49 |
| Underflow | 345 | 50.59 | 4.05 | 17.95 | 7.36 | 9.49 | 11.01 | 76.97 | 48.69 | 54.51 |
| Calc. Head | 682 | 100.00 | 18.60 | 11.80 | 7.65 | 8.81 | 100.00 | 100.00 | 100.00 | 100.00 |
| 35 PSI | | | | | | | | | | |
| Overflow | 352 | 49.44 | 31.7 | 5.33 | 7.58 | 7.46 | 87.32 | 23.00 | 50.11 | 44.01 |
| Underflow | 360 | 50.56 | 4.50 | 17.45 | 7.38 | 9.28 | 12.68 | 77.00 | 49.89 | 55.99 |
| Calc. Head | 712 | 100.00 | 17.95 | 11.46 | 7.48 | 8.38 | 100.00 | 100.00 | 100.00 | 100.00 |
| 40 PSI | | | | | | | | | | |
| Overflow | 345 | 47.33 | 31.6 | 6.10 | 6.86 | 6.93 | 86.58 | 22.93 | 44.14 | 39.57 |
| Underflow | 384 | 52.67 | 4.40 | 18.42 | 7.80 | 9.51 | 13.42 | 77.07 | 55.86 | 60.43 |
| Calc. Head | 729 | 100.00 | 17.27 | 12.59 | 7.36 | 8.29 | 100.00 | 100.00 | 100.00 | 100.00 |

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Table 11
Cont.d

IV Density : 12 % Solids pH = 9.5

| | gm wt | % wt | Assays C | Mo | Cu | Fe | % Distribution C | Mo | Cu | Fe |
|---|---|---|---|---|---|---|---|---|---|---|
| 25 PSI | | | | | | | | | | |
| Overflow | 534 | 58.42 | 30.2 | 8.25 | 8.03 | 8.40 | 91.78 | 41.18 | 57.67 | 51.80 |
| Underflow | 380 | 41.58 | 3.80 | 16.76 | 8.28 | 10.98 | 8.22 | 58.82 | 42.32 | 48.20 |
| Calc. Head | 914 | 100.00 | 19.22 | 11.85 | 8.13 | 9.47 | 100.00 | 100.00 | 100.00 | 100.00 |
| 30 PSI | | | | | | | | | | |
| Overflow | 566 | 56.77 | 29.8 | 7.51 | 7.01 | 6.97 | 91.58 | 39.15 | 53.91 | 47.54 |
| Underflow | 431 | 43.23 | 3.60 | 15.33 | 7.87 | 10.10 | 8.42 | 60.85 | 46.09 | 52.46 |
| Calc. Head | 997 | 100.00 | 18.47 | 10.89 | 7.38 | 8.32 | 100.00 | 100.00 | 100.00 | 100.00 |
| 35 PSI | | | | | | | | | | |
| Overflow | 574 | 55.57 | 30.4 | 7.87 | 7.45 | 7.68 | 90.70 | 34.00 | 55.24 | 49.94 |
| Underflow | 459 | 44.43 | 3.90 | 19.11 | 7.55 | 9.63 | 9.30 | 66.00 | 44.76 | 50.06 |
| Calc. Head | 1033 | 100.00 | 18.63 | 12.86 | 7.49 | 8.55 | 100.00 | 100.00 | 100.00 | 100.00 |
| 40 PSI | | | | | | | | | | |
| Overflow | 590 | 53.64 | 31.5 | 7.30 | 7.42 | 7.74 | 89.23 | 31.44 | 52.40 | 48.50 |
| Underflow | 510 | 46.36 | 4.40 | 18.42 | 7.80 | 9.51 | 10.77 | 68.56 | 47.60 | 51.50 |
| Calc. Head | 1100 | 100.00 | 18.94 | 12.45 | 7.60 | 8.56 | 100.00 | 100.00 | 100.00 | 100.00 |

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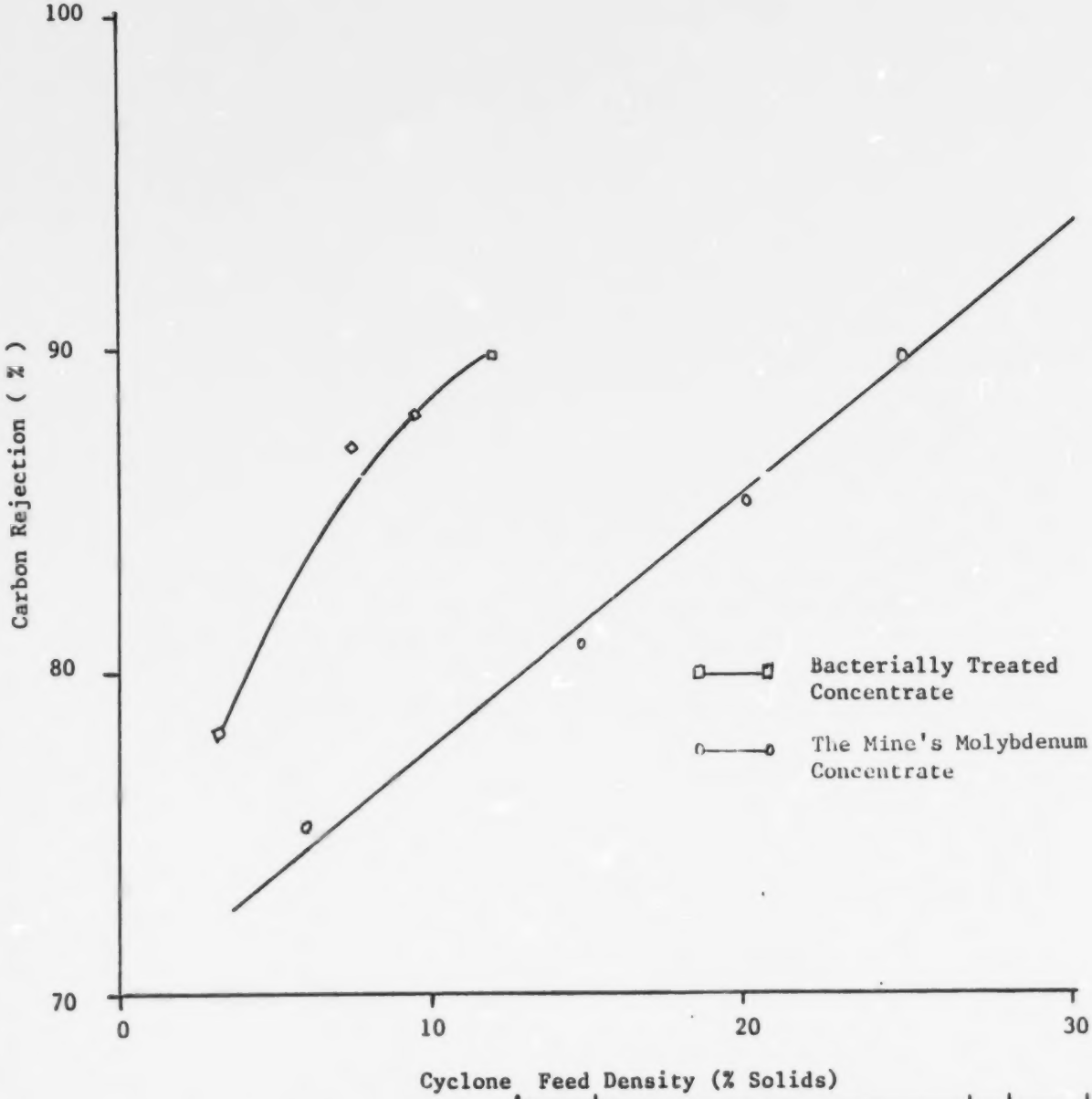
Figure 14 a

Carbon Rejections Obtained from Cyclone Runs from:

i) A Bacterially Treated Molybdenum Ore Concentrate and

ii) The Mine's Cycloning Data




| REV. No. | REVISION | BY | DATE | CHK | DATE |
|---|---|---|---|---|---|
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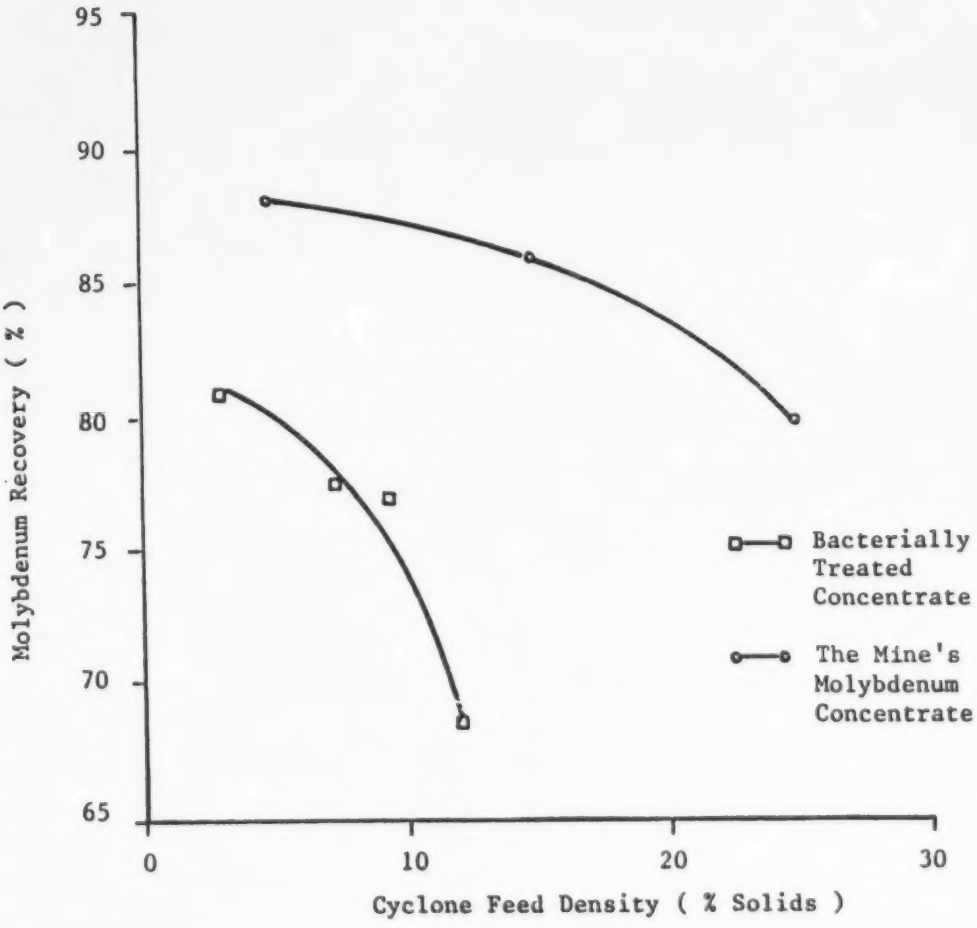
WITTECK DEVELOPMENT INC.

| REF. DWG. No. | TITLE | TITLE | BY | CHK | DATE |
|---|---|---|---|---|---|
| | | DWG. No. | PROJECT No. | | REV. |

Figure 14b

Molybdenum Recoveries Obtained
From Cylone Runs From:

1. A Bacterially Treated Molybdenum Ore Concentrate And
2. The Mine's Cycloning Data



## 5.9 Mineralogical Examination of Polished Sections of Treated and Untreated Molybdenum Concentrate

Comparison of the mineralogical examinations from the bacterially treated and untreated concentrate revealed no conclusive evidence of visual alterations in the mineral structures. In both treated and untreated samples direct associations of a carbonaceous species and molybdenite were noted.

A variety of mineral species, both free and associated with other minerals, were observed during the reconnaissance scanning of the polished sections. Figures 15 to 24 show the type of mineral species present in the molybdenum concentrate.

Figures 15 to 17 show typical free mineral species. The major minerals present were molybdenite, carbonaceous gangue (gilsonite), pyrite, and chalcopyrite. Graphite and sphalerite have also been observed in minor amounts.

Middling particles were less common than free minerals. Figure 20 illustrates a middling association between molybdenite and chalcopyrite. Figure 21 (from the untreated control) shows the association of molybdenite and gilsonite.

The observations suggest the hypothesis that mineral separation problems could be due to non-selective "sticking". Figure 23 also shows a middling association between chalcopyrite and carbon. Figure 24 displays inclusions of sphalerite and chalcopyrite within molybdenite.

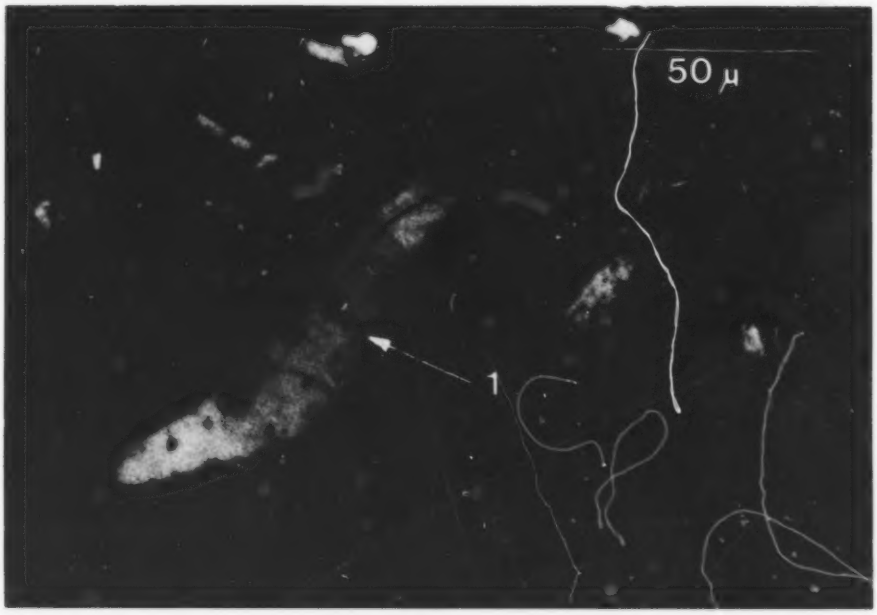



Figure 15
500X RL
Free particles of molybdenite (1)

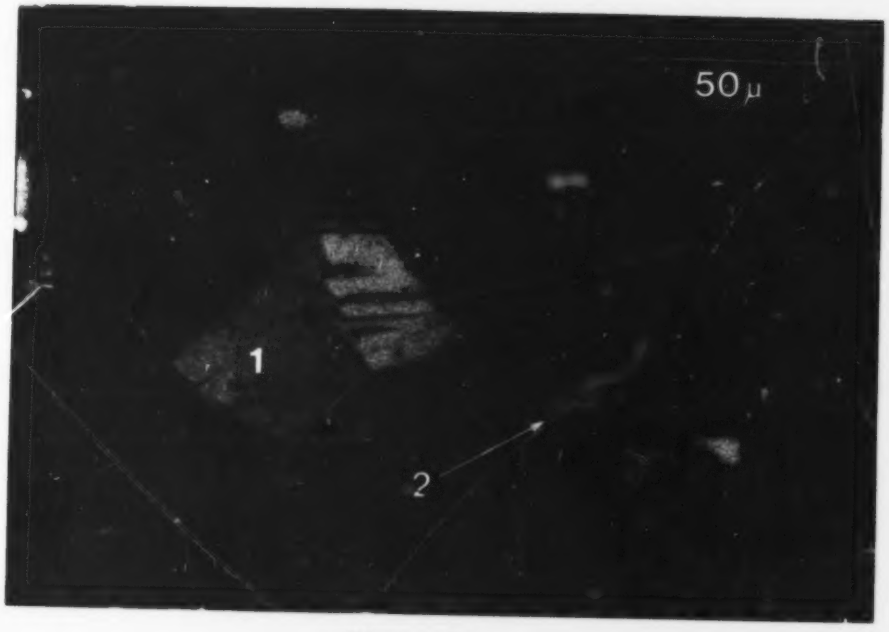



Figure 16
500X RL
Free particles of molybdenite (1) and chalcopyrite (2).

Figure 17.
200 X RL
Angular free particles of molybdenite (1) and pyrite (2).

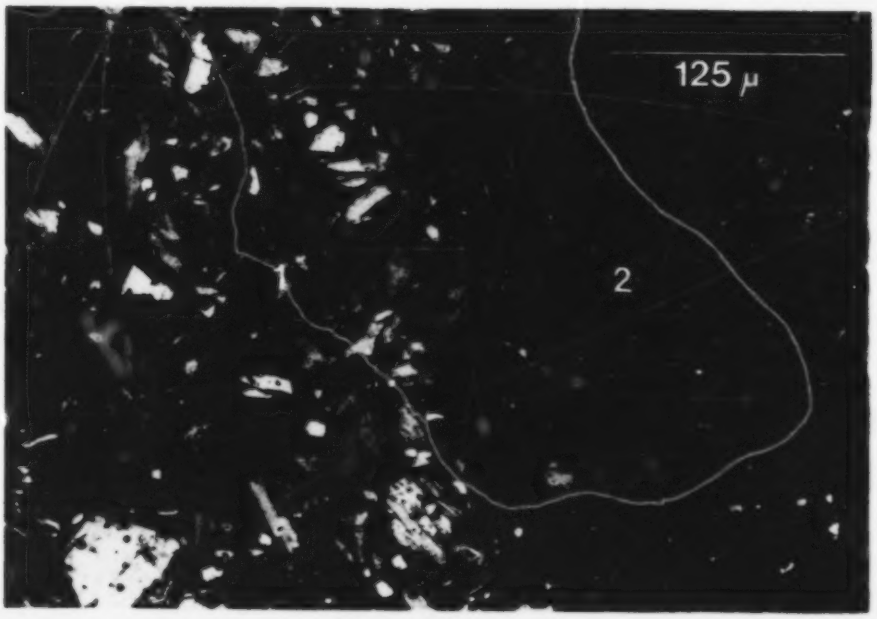



Figure 18.
200 X RL
Overview of molybdenite particles (1)
and gilsonite particles (2).

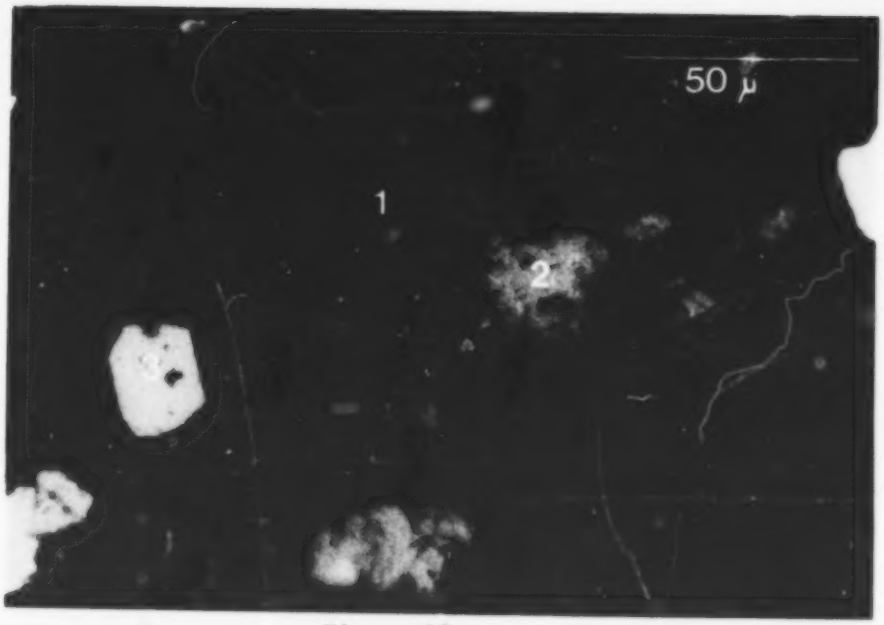



Figure 19.
500 X RL
Angular free particles of graphite (1),
molybdenite (2) and pyrite (3).

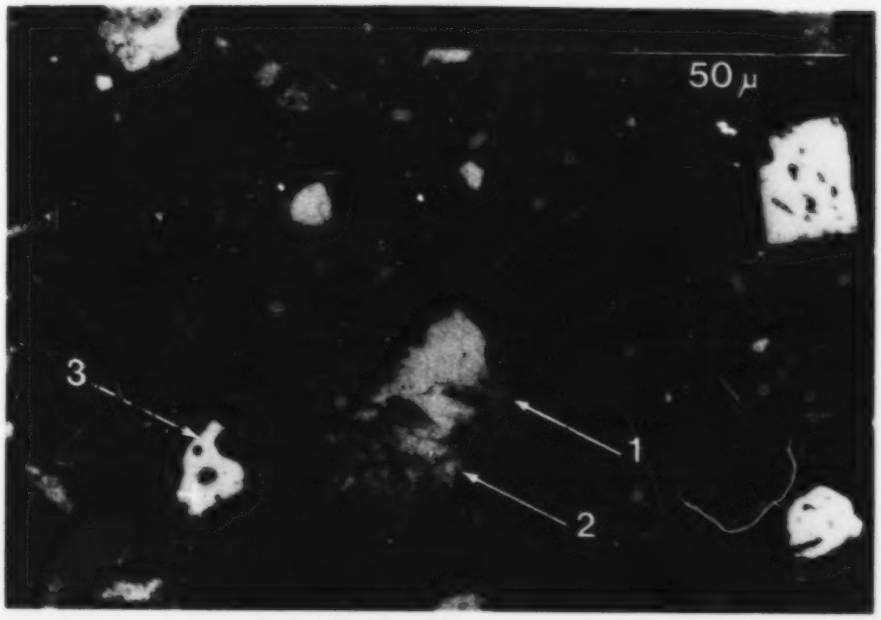



Figure 20.
500 X RL
Middling association between chalcopyrite (1) and molybdenite (2). Free pyrite (3) is also shown.

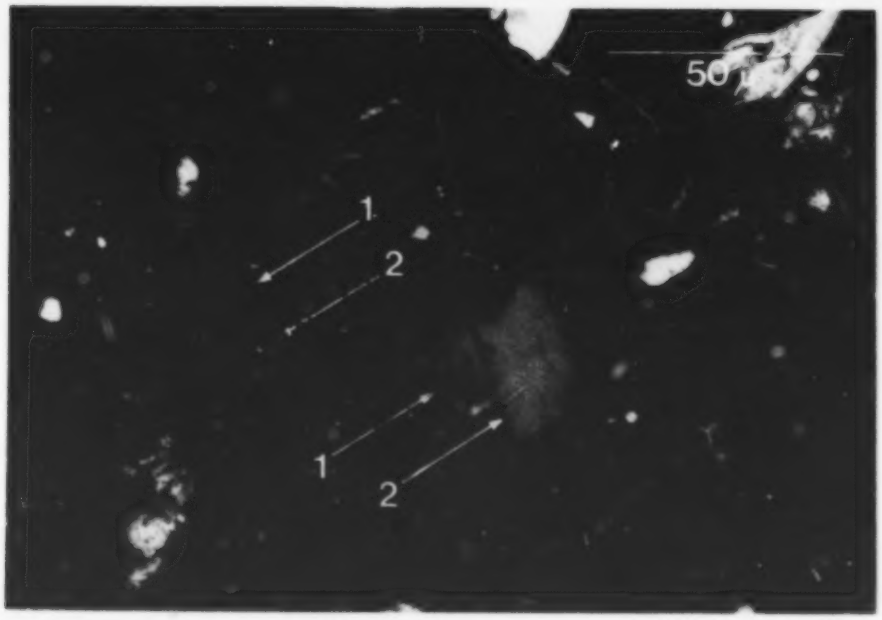



Figure 21.
500 X RL
Mineral associations between gilsonite (1) and molybdenite (2).



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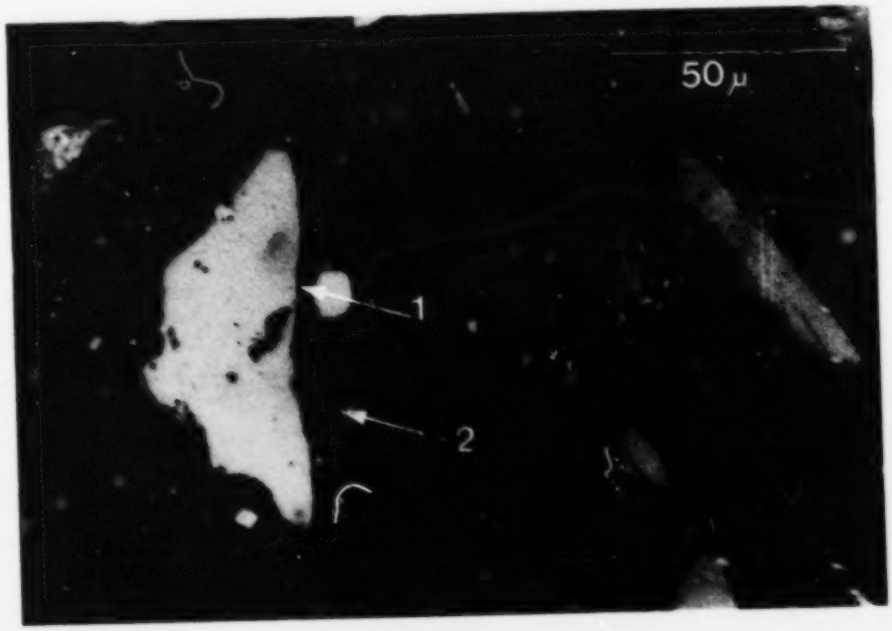


Figure 22.
500 X RL
Middling association between chalcopyrite (1)
and molybdenite (2).

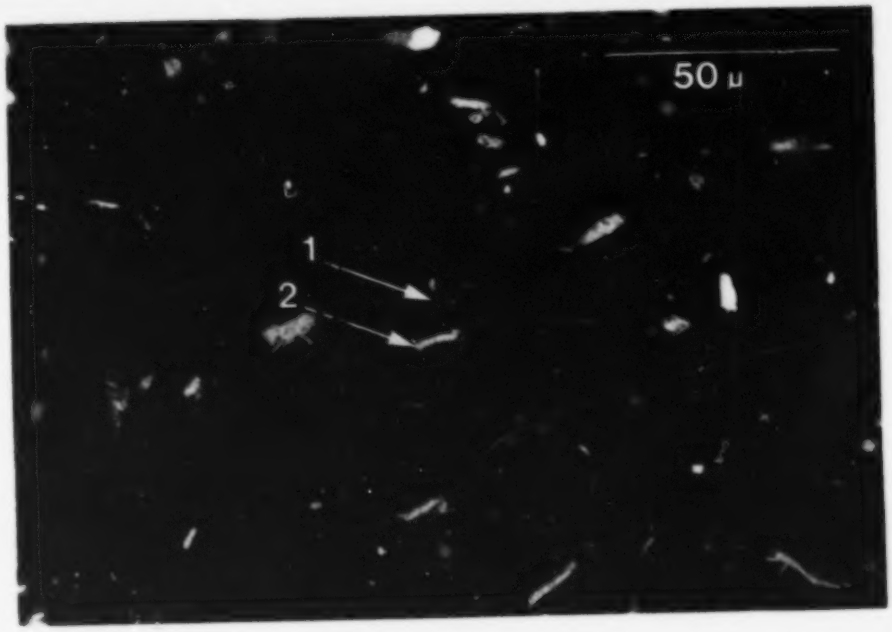


Figure 23.
500 X RL
Middling association between gilsonite (1)
and chalcopyrite (2).

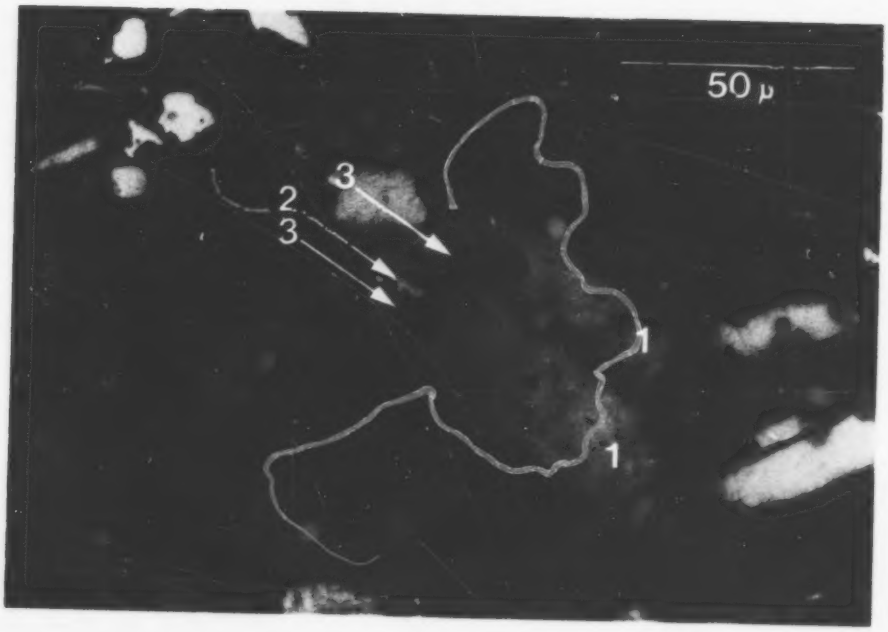


Figure 24.
500 X RL
Middling association of chalcopyrite (2) and sphalerite (3) within molybdenite (1). Associations of this kind are rare.

## 5.10 Observations of Bacterial Growth and Hydrocarbon Degradation During the Treatment of Molybdenum Concentrate in a Semi-Continuous Decantation Mode

In the batch bacterial treatment of the molybdenum concentrate, there was minimal loss of carbon in the system due to the bacterial oxidation and/or degradation. This indicated that the system would remove carbon much more effectively if the bacterial solution was decanted to remove biomass and suspended particulate carbon. Consequently, a semi-continuous ore concentrate treatment system was set up to assess the removal of carbon by bacterial degradation and subsequent surfactant production. A table of values obtained from the experiment is summarized in Table 12.

Following 24 hours of treatment, a surfactant was produced by the bacteria in the treatment flask with the same qualitative properties as the surfactant produced in the pilot scale run. Assays of suspended particles and the head assay from the agitated reactor vessel were determined. The results, shown in Table 13, indicate that a considerable amount of the carbon was concentrated in the suspended fraction, which contained from 0.2 to 1% solids, depending on the settling time allotted for the slurry. In a negative control flask (no bacteria), no noticeable surfactant was produced and all of the solids settled in about 15 minutes.

A protozoan was found in the semi-continuous treatment flask after 7 days of treatment. The protozoan was a mastigophoran with two unequal flagella. The cell was 8-20 m wide with a spherical body. No chloroplasts were observed. Tentative indentification of this protozoan was Bodo sp.

Table 12

pH, BACTERIAL COUNTS (CFU/mL), CARBON ASSAYS AND Eh MEASUREMENTS FROM A SEMI-CONTINUOUS TREATMENT OF MOLYBDENUM CONCENTRATE. NUTRIENT SOLUTION WAS REPLACED EVERY 24 HOURS

| Day | pH | Eh | CFU/mL | %C | Notes |
|---|---|---|---|---|---|
| 0 | 7.1 | - | $3x10^{0}$ | 22.7 | |
| 1 | 7.48 | -63 mV | $1x\ 10^{9}$ | - | |
| 2 | 7.26 | - | $6.1x10^{8}$ | - | |
| 4 | 7.28 | -54 mV | $1.2x10^{8}$ | 21.7 | |
| 5 | 7.46 | -55 mV | $2.3x10^{8}$ | 17.2 | |
| 6 | 7.33 | -27 mV | $8.7x10^{7}$ | 19.8 | |
| 7 | 7.22 | +40 mV | - | 18.3 | Protozoa Present |
| 8 | 7.35 | +43 mV | - | 17.6 | |
| 11 | 7.41 | +47 mV | $8.2x10^{8}$ | 17.6 | |
| 12 | 7.38 | +43 mV | $3.3x10^{8}$ | 17.2 | |
| 13 | 7.53 | +52 mV | - | 17.0 | |
| 14 | 7.50 | +47 mV | - | 16.8 | |

Table 13

ASSAYS OF CARBON AND MOLYBDENUM FROM PARTICLES REMAINING IN THE SEMI-CONTINUOUS TREATMENT FLASK AFTER 1 HOUR OF SETTLING AND HEAD ASSAY WHEN AGITATED

| | %C | %Mo |
|---|---|---|
| Head Assay | 18.8 | 12.5 |
| Assay of Suspended Particles | 40.5 | 3.5 |

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## 6.0 DISCUSSION

The results of this study demonstrated that hydrocarbon degrading microorganisms have easily acclimated to hydrocarbon species present in the Mine's hydrocarbon-rich molybdenum ore concentrate.

The mixed population of microorganisms was composed of several species and has survived several stages of subculturing to remove undesirable microorganisms and inefficient hydrocarbon degraders. The mixed population of bacteria was isolated from activated sludge reactors from a waste petroleum processing plant. The mixed population, composed of 5 strains, degraded the asphalt and light oil fractions of the molybdenum concentrate very effectively when a slurry of up to 15% solids was supplied with nitrogen, phosphorous, sulfate, magnesium, iron and yeast extract. The mixed population was composed of two Pseudomonas, one Flavobacterium, one Mycobacterium, and one Arthrobacter. It was not known whether these strains can survive in the absence of one another on the hydrocarbon substrate. It is assumed that a complex enzymatic system has developed due to the stability of this mixed culture, and that synergistic and co-oxidational roles are present.

Table 7 indicates that at least 15% of the hydrocarbon in the rougher and final ore concentrates was extractable with organic solvents. It was assumed that the benzene soluble fraction contained the aliphatic hydrocarbons, the aromatic hydrocarbon and the asphaltene hydrocarbons. The remaining hydrocarbons are presumed to be insoluble solid hydrocarbons, graphitic carbon and/or carbonate carbon. It is most likely that the "sticky" benzene-soluble fraction was the major contributor to the hydrocarbon-molybdenum separation problems. It is also believed that the majority of the bacterial carbon and energy sources are from this fraction.

Infrared spectrophotometric analyses of the bacterially treated samples indicated that oxidation of the organically extracted hydrocarbon material was present (Figures 3 and 4). Hydrocarbon oxidation was indicated although we could not determine the oxidized hydrocarbon species through I.R. The presence of the O-H functional groups indicates the oxidation of the hydrocarbon species.

The gas chromatograms of the treated and untreated tar-sand samples revealed changes in hydrocarbon species due to the bacterial treatment. The bacterially treated tar-sand sample caused a shift to the left in the aromatic and polar hydrocarbons. Saturated hydrocarbons were consumed by the bacteria while there was no saturate hydrocarbon degradation in the negative control flask. The gas chromatographic studies demonstrated that the bacterial treatment altered the total composition of the organically extractable hydrocarbon material.

The results obtained from the tar-sand studies clearly demonstrated that the bacteria effectively stripped the tar from the tar-sand. The stripping of the tar from the tar-sand is due to three possible mechanisms: (i) mechanical action due to shaking, (ii) bacterial hydrocarbon degradation and (iii) by the action of a bacterial surface-active agent.

The mechanical action of stripping tar from tar-sand is minor in relation to the bacterial action. Minor separation of agglomerations of tar-sand particles were evident when comparing the unshaken control flask (Figure 5) to shaken control flask (Figure 6).

Bacterial hydrocarbon degradation cannot be determined visually by inspecting the tar sands. Gas chromatographic analyses demonstrated that degradation of high molecular weight hydrocarbons to the lower molecular weight hydrocarbons has occurred. It was assumed that the bacterial degradation of high molecular weight hydrocarbons renders them less viscous than their original form.

The production of surface-active agents by the mixed bacterial culture is believed to cause the emulsification of the hydrocarbon material, a very important factor involved in the removal of tar from the tar-sand. It is suggested that the action of mechanical shaking and bacterial surfactant production causes the removal of tar from tar-sand. The emulsification of hydrocarbons would also promote the respiratory activity of the cells by enabling easier access of hydrocarbons via the aqueous phase.

The demonstration of bacterial surfactant production was most noticeable when the bacterial culture was propagated on the final molybdenum concentrate. It is not known if more than one surfactant was produced by different bacterial species, although it is likely, since Pseudomonas and Arthrobacter are both known to produce surfactants.

The semi-continuous bacterial treatment of molybdenum concentrate showed that surfactant production was clearly evident. The assays of suspended particles versus slurried particles in Table 13 shows the effect of the surfactant production on the suspension of particulate carbon. It is suggested that bacterial surfactant production be examined in the future as a means of decanting carbon from the ore concentrate. Optimization of a continuously fed and decanted system will be utilized to suspend the maximal amount of carbon with a minimal loss of molybdenite.

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From the preliminary flotation and vanning tests of the bacterially treated ore samples, it was not possible to conclude that the bacterial treatment has had any effect on the separation of carbon from molybdenite. The metallurgical test work was abandoned when it was discovered that cycloning proved more efficient when separating hydrocarbon from molybdenum.

The one-stage cyclone test of the bacterially treated final molybdenum concentrate showed increased rates of carbon rejection as compared to the Mine's previous one-stage cyclone runs. Molybdenum recoveries and carbon rejections can be further improved if a two-stage cyclone set-up is constructed in future runs.

Mineralogical examinations of the minerals in the treated and untreated final molybdenum ore concentrates did not show distinct differences in mineral associations. If the "sticky" bituminous fraction of the carbonaceous material is causing non-selective sticking of ore particles, this rimming texture may be better observed by thin section analysis of the concentrates.

## 7.0 CONCLUSIONS AND RECOMMENDATIONS

Bacteria acquired from waste petroleum activated sludge plants were quickly and effectively acclimated to the hydrocarbon species in the Mine's molybdenum ore concentrates. Bacteria cultivated on the ore concentrates in mixed culture degraded hydrocarbon species at both bench and pilot scale level. The most predominant bacteria belonged to the genus Pseudomonas sp. Bacterial species including Mycobacterium sp., Arthrobacter sp. and Flavobacterium sp. were also present. Protozoa were also observed in some cultures with Bodo sp. as the most predominant protozoan.

Cyclone trials of bacterially treated molybdenum ore concentrate gave better carbon rejections than the Mine's previous runs, although molybdenum recoveries were less efficient. It is recommended that future cycloning tests be performed on the treated ore concentrates at higher percent solids and in a two-stage configuration so as to maximize molybdenite recoveries and carbon rejections. Mineralogical examinations of polished sections of treated and untreated ore concentrates identified several carbon-molybdenum middlings particles but it was not known how the two mineral species were associated. It is recommended that thin section analyses of treated and untreated ore concentrates be performed to observe the optical changes in the hydrocarbon species due to bacterial treatment.

The production of a surfactant through the bacterial treatment caused the suspension of particles which contained high percentages of carbon. The use of a bacterially produced surfactant to remove carbon by decantation may be used as a pretreatment to further metallurgical tests.

Bench scale work with the hydrocarbon oxidizing bacteria should be continued in the next phase of work. The construction of continuously-feeding and decanting reactors must be carried out in order to optimize the degradation and removal of hydrocarbon species in the ore concentrates.

Prospective methods of decanting carbon from a continuous phase decantation system need to be studied in detail to determine if the methods are feasible and economical.

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## 8.0 ACKNOWLEDGEMENTS

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# APPENDIX I

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# APPENDIX II

## GAS CHROMATOGRAPHIC SPECTRA

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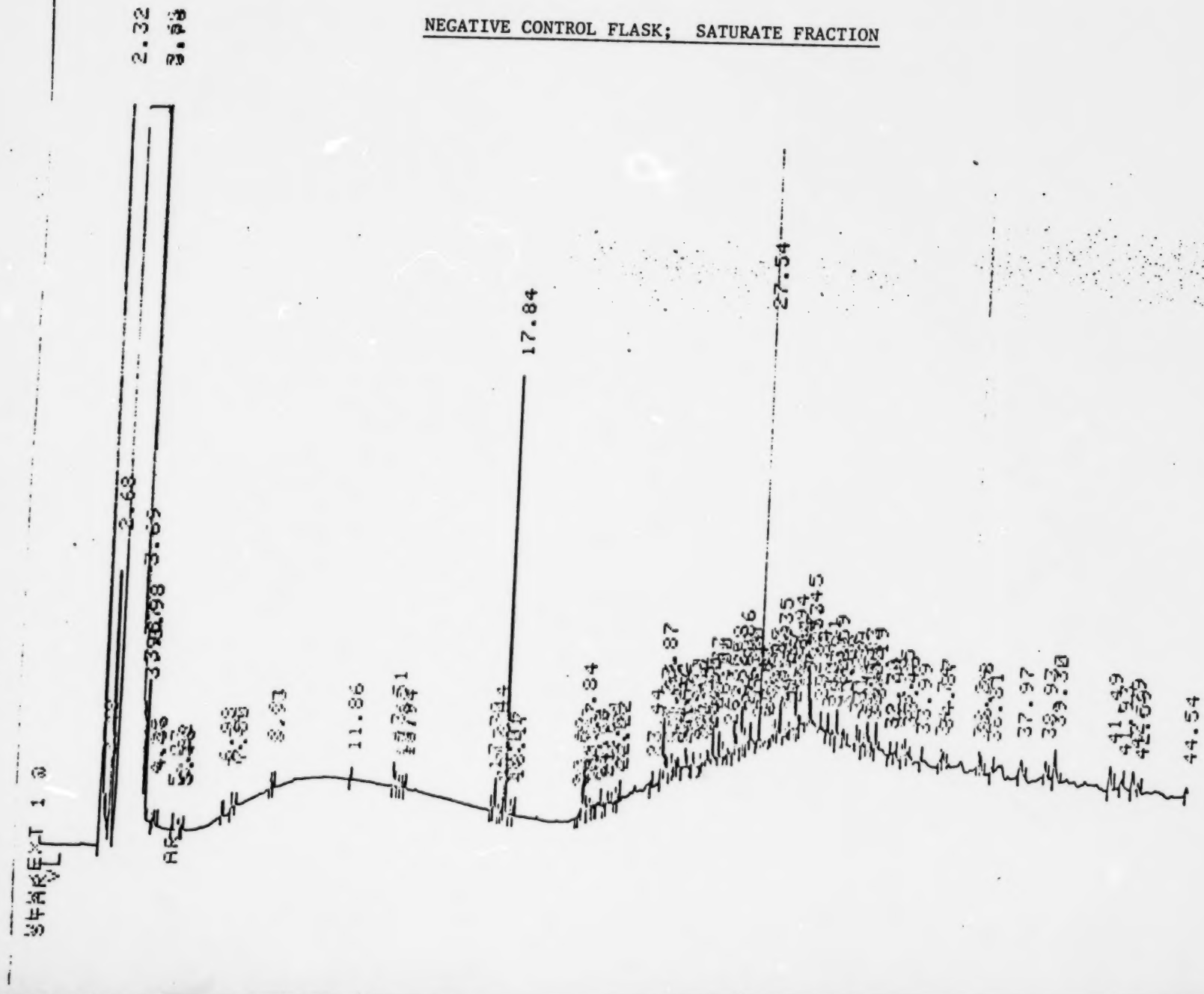
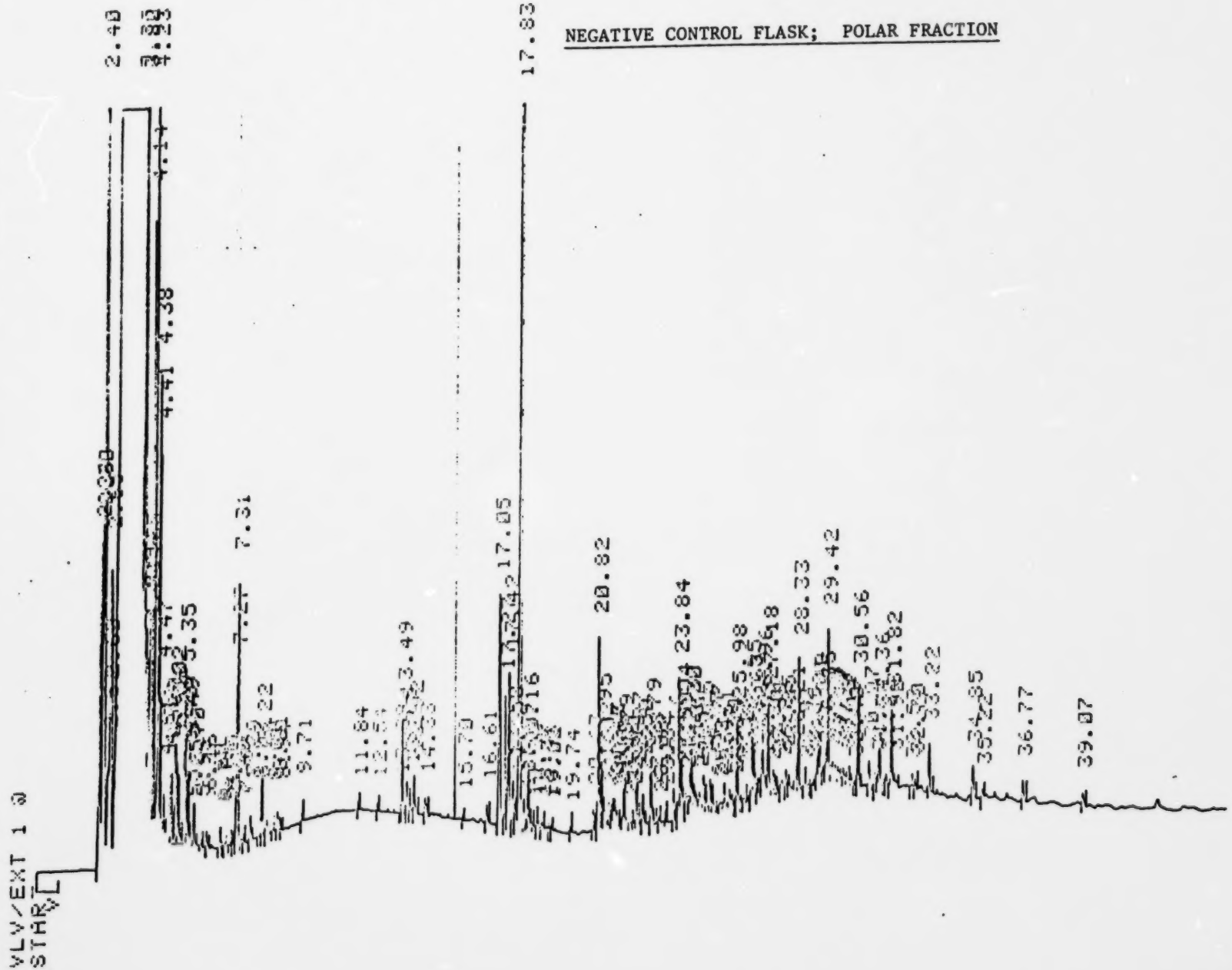
NEGATIVE CONTROL FLASK; SATURATE FRACTION

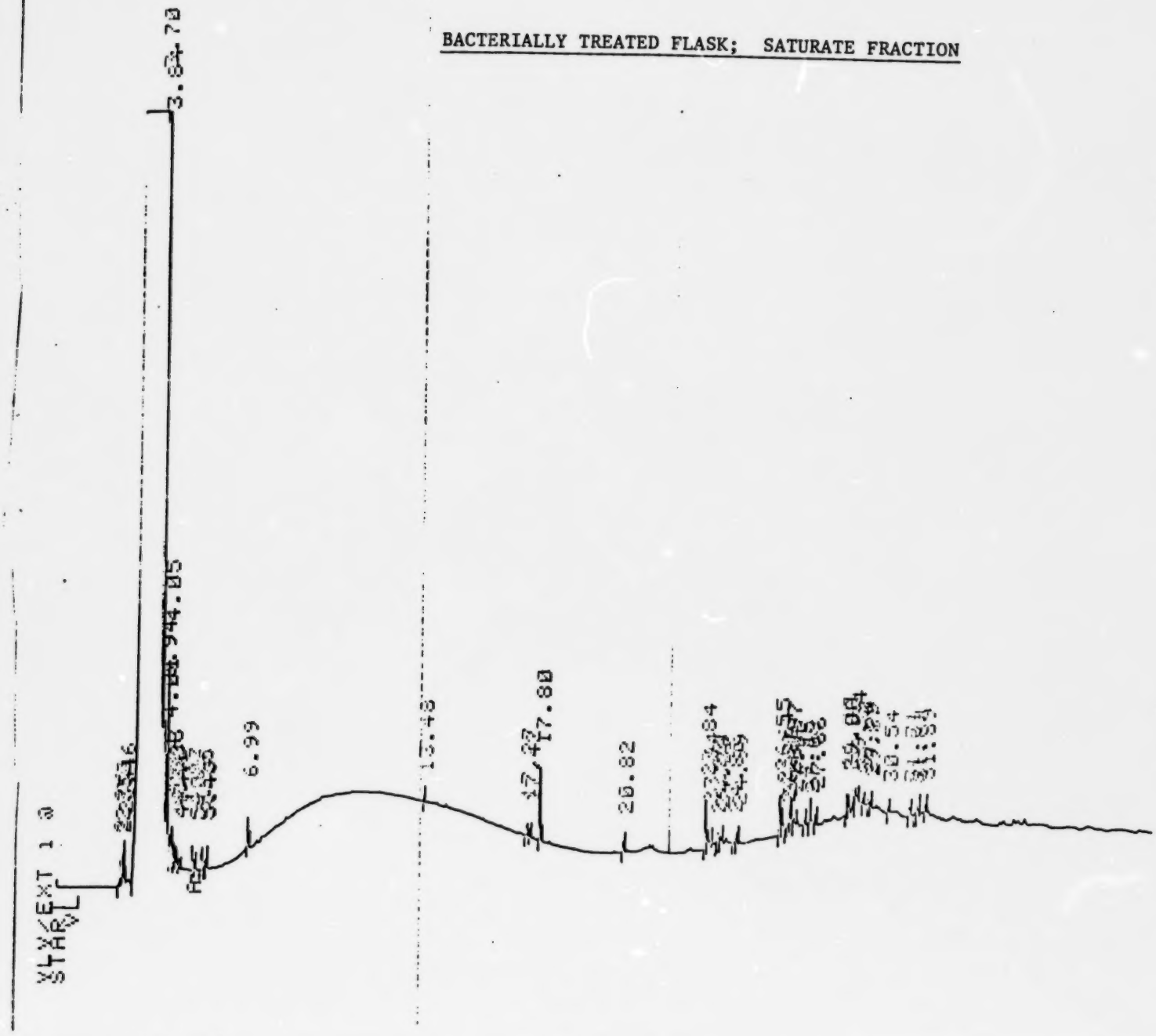


NEGATIVE CONTROL FLASK; AROMATIC FRACTION

NEGATIVE CONTROL FLASK; POLAR FRACTION



BACTERIALLY TREATED FLASK; SATURATE FRACTION



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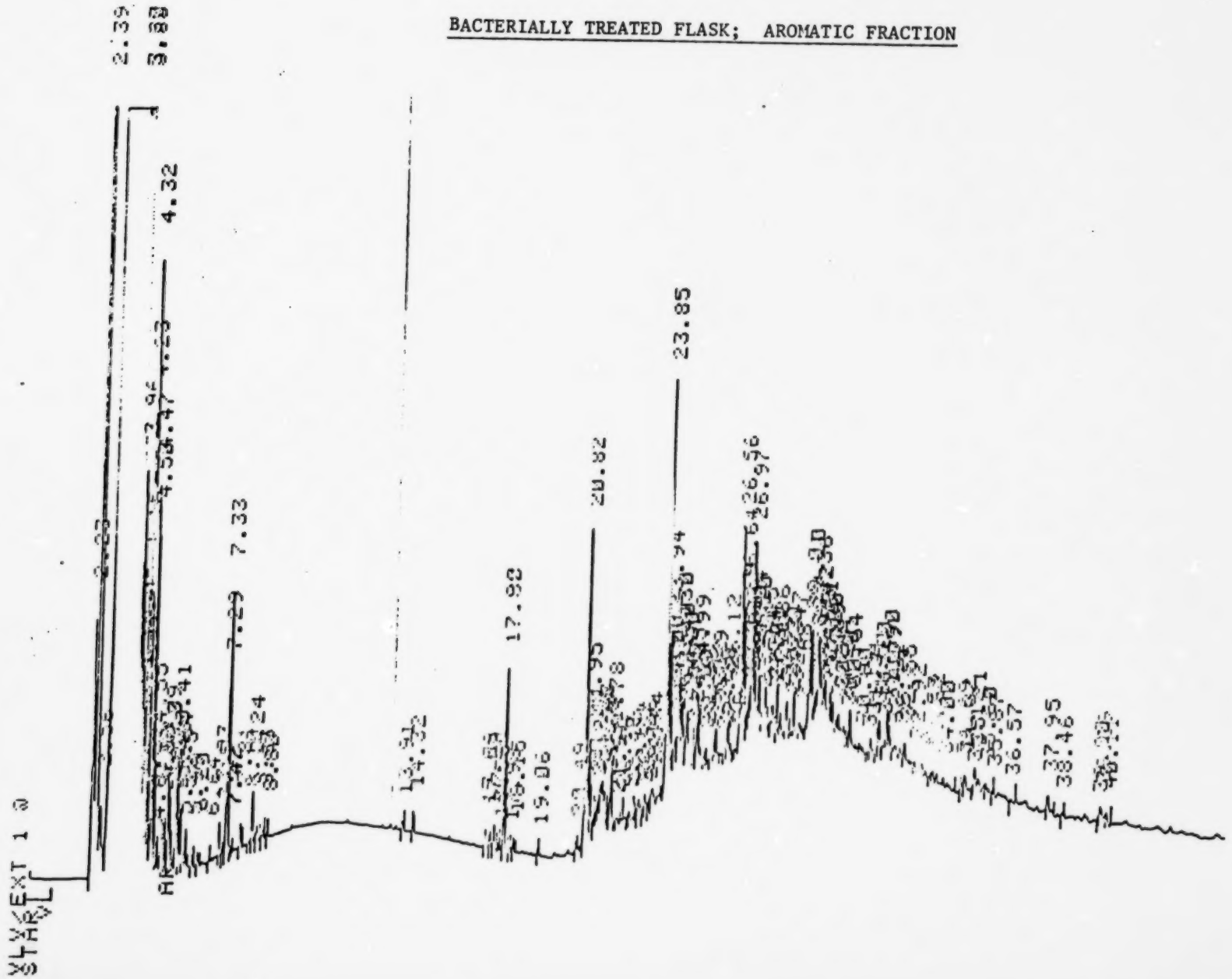

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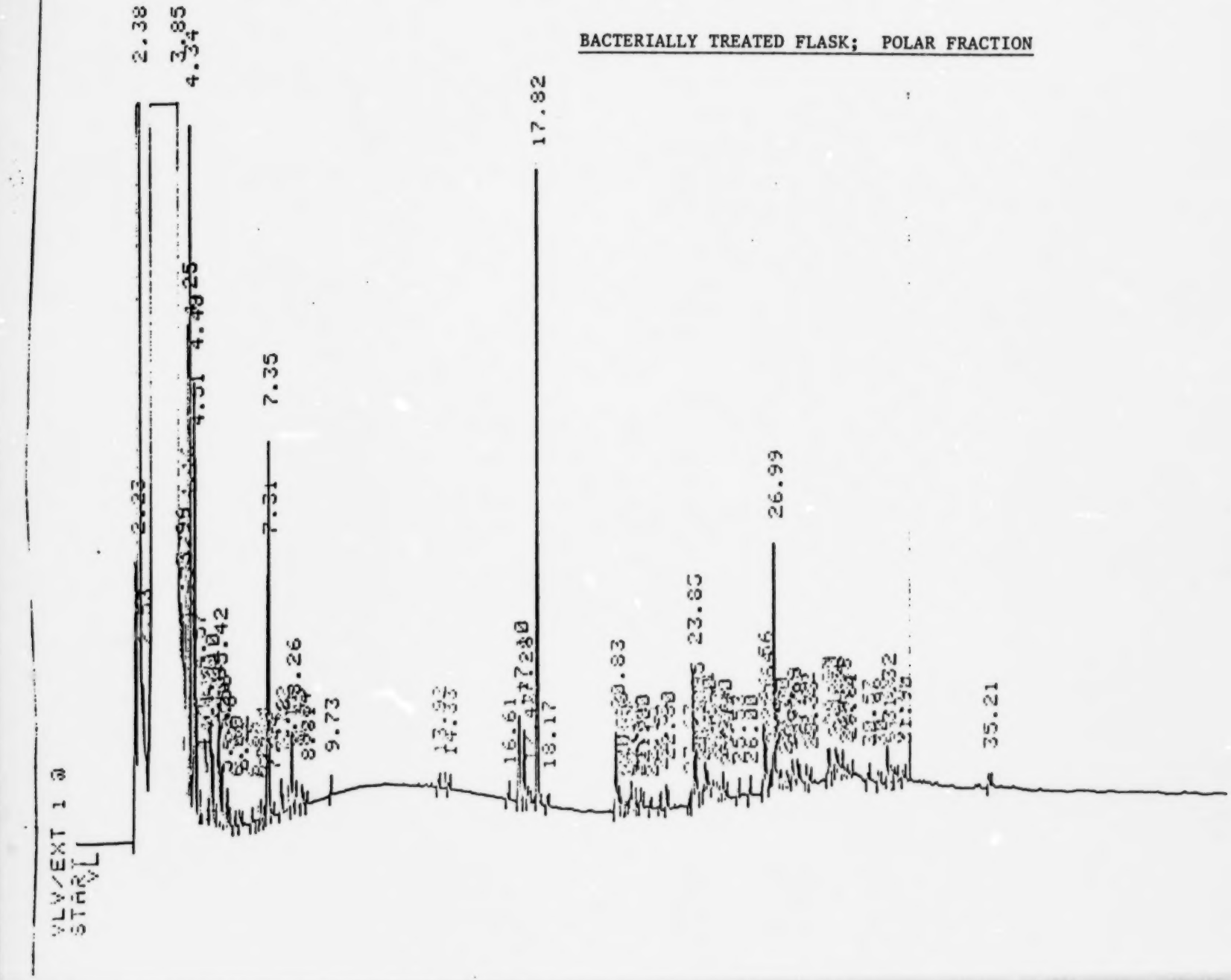
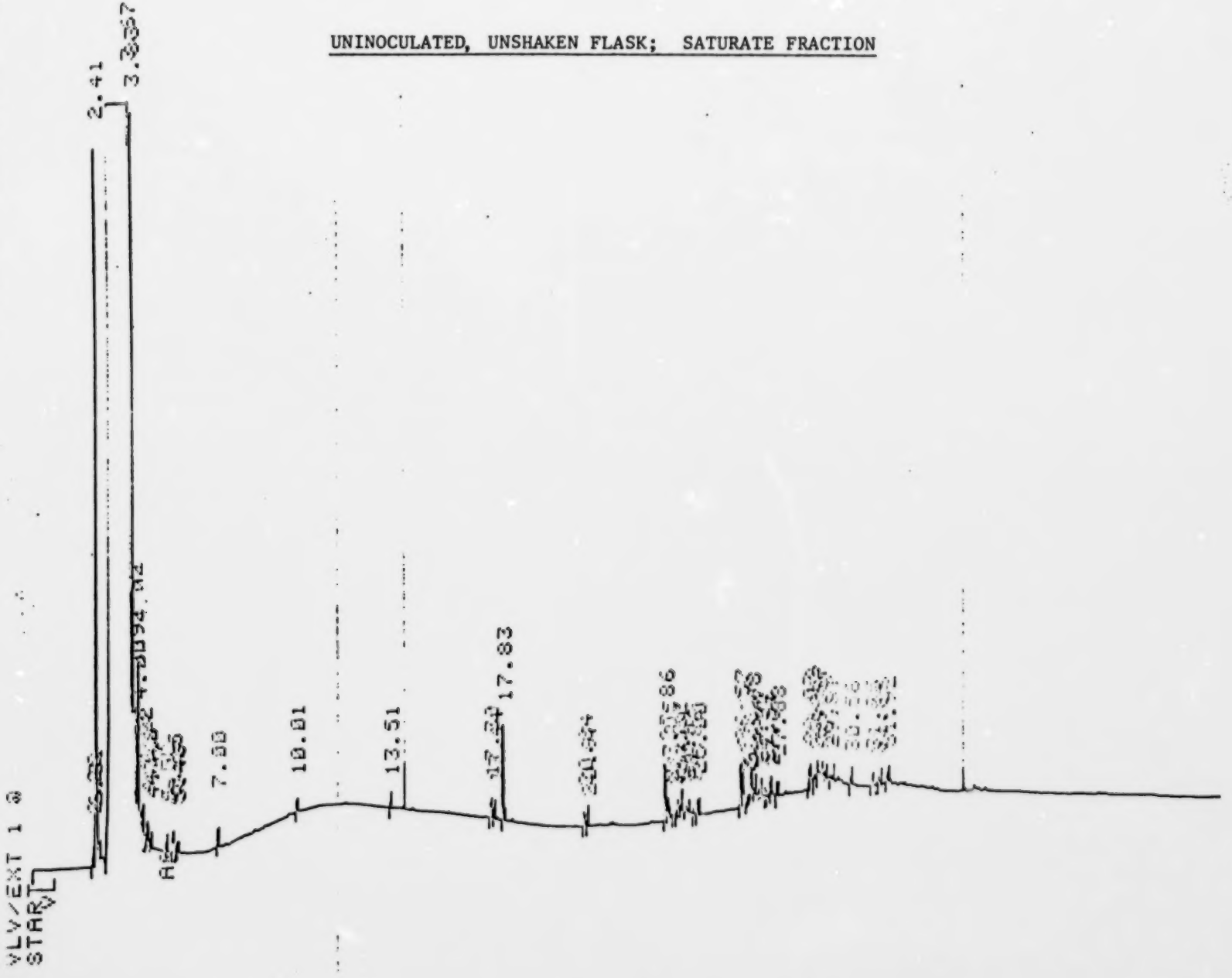
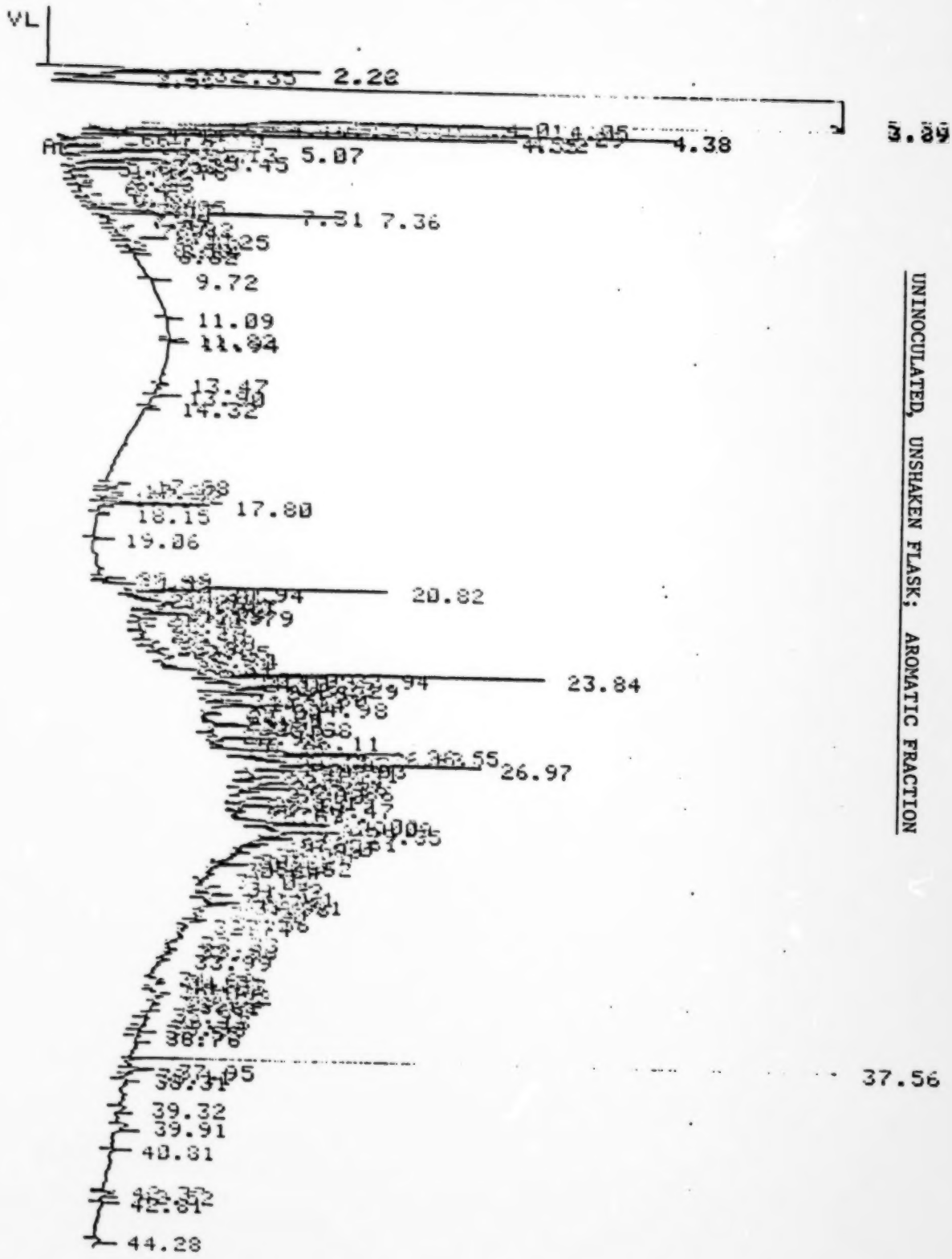


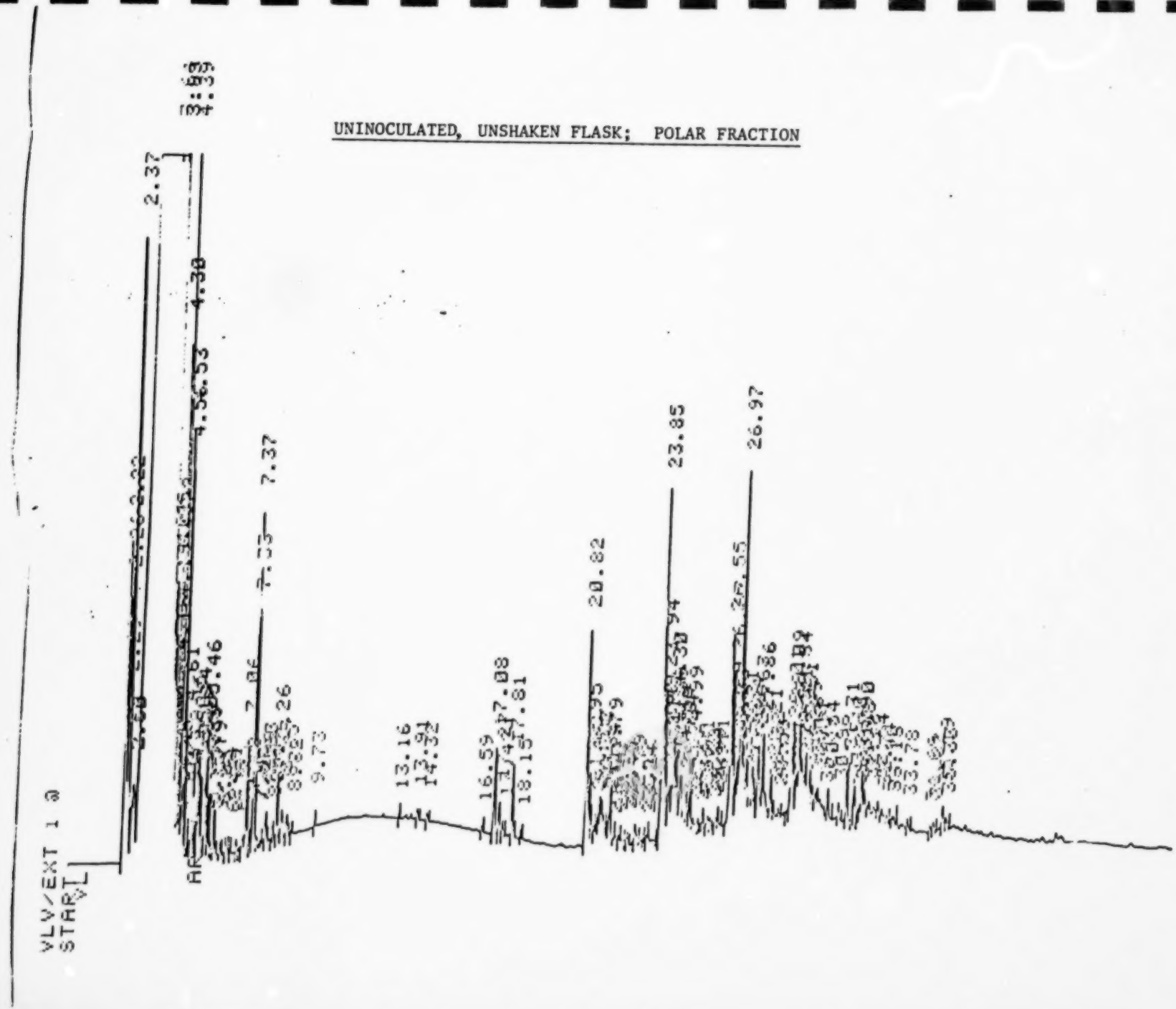
UNINOCULATED, UNSHAKEN FLASK; SATURATE FRACTION

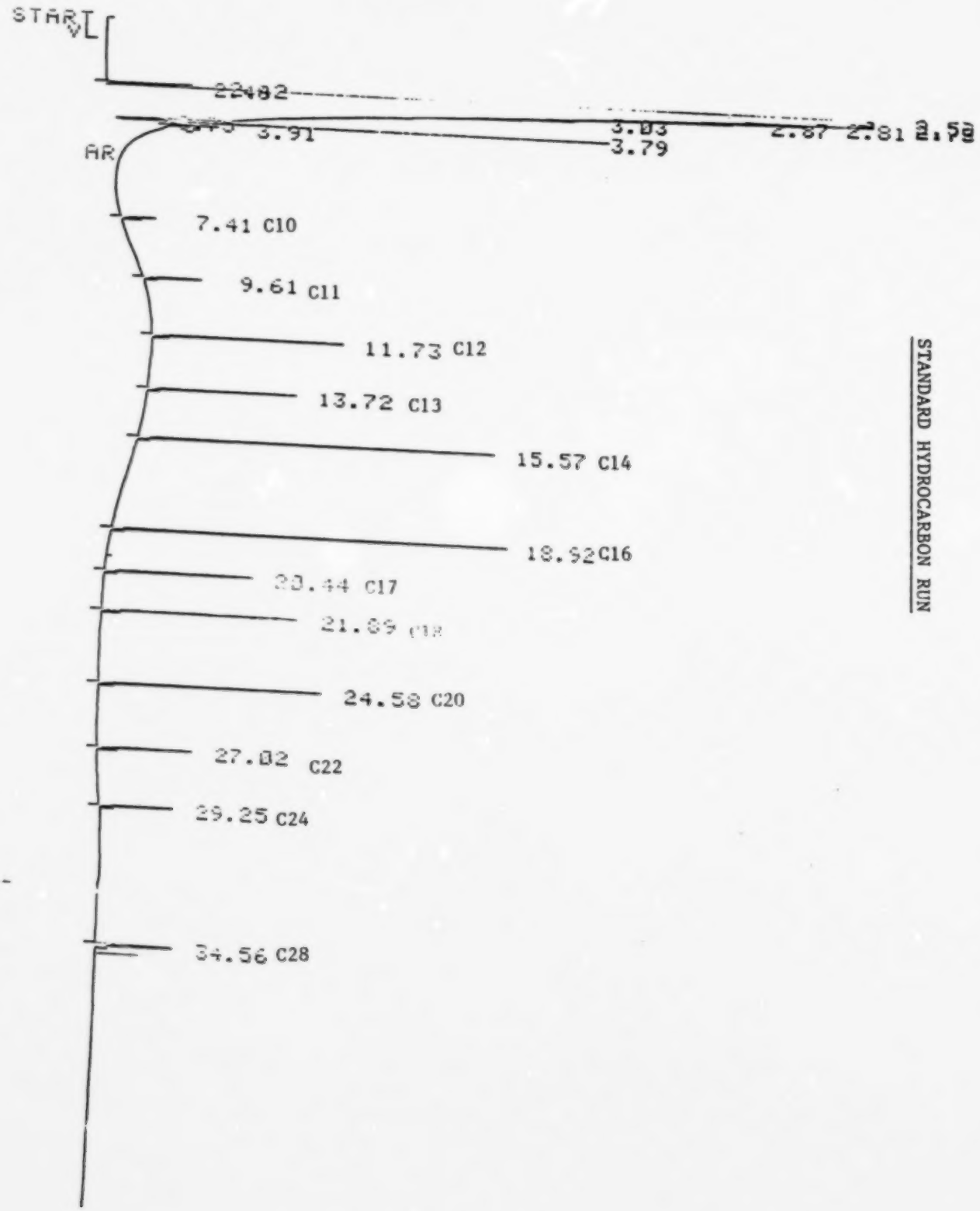




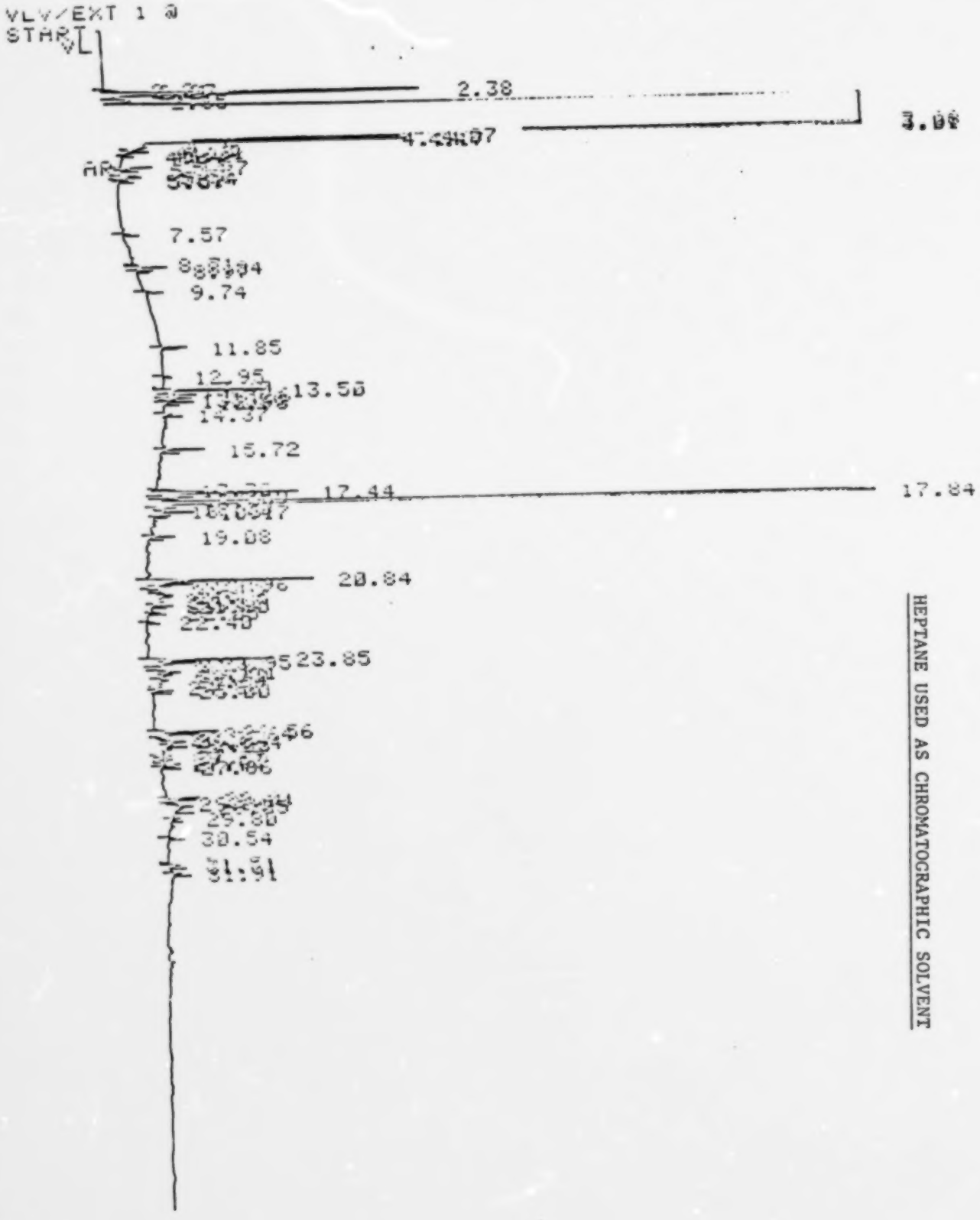

UNINOCULATED, UNSHAKEN FLASK; AROMATIC FRACTION

UNINOCULATED, UNSHAKEN FLASK; POLAR FRACTION






STANDARD HYDROCARBON RUN




HEPTANE USED AS CHROMATOGRAPHIC SOLVENT